THE MECHANISM OF IRRADIATION INDUCED TEXTURAL CHANGES IN TOMATO FRUIT (*LYCOPERSICON ESCULENTUM*)

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In memory of my mother
And to my father

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LIST OF ABBREVIATIONS

AIS	alcohol-insoluble solids
BP	buffered phenol
CDTA	1,2-cyclohexylenedinitrilotetraacetic acid
dI	distilled water
EtOH	ethanol
M_r	molecular weight
PG	polygalacturonase
PME	pectinmethylesterase

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'Sunny' tomato fruit at mature-green and pink stages of development were subjected to ionizing irradiation from gamma-rays or X-rays at various doses Additionally, alcohol-insoluble solids (AIS) derived from 'Sunny' tomato fruit of both maturity classes salt-extractable proteins, pure polygalacturonase (PG), commercial preparations of pectin, polygalacturonic acid and cellulose and PG antisense 'Flavr Savr' mature-green tomato fruit were irradiated with gamma rays at various doses

An immediate softening was observed following irradiation treatments. Lower levels of total uronic acid in the high irradiation treatments, along with increased levels of H_2O-soluble polyuronides in both irradiation dose treatments were observed in the irradiated fruit of both maturity classes. Irradiated fruit also exhibited downshifts in the M_r of soluble polyuronides. Enhanced electrolyte efflux was apparent in irradiated fruit mainly in fruit at the pink stage of development. A significant reduction of PG activity along with increased activities of PME and β-galactosidase immediately following irradiation were followed by reductions at later stages in both maturity stages. Losses in total uronic acid and increased solubility of pectic fractions occurred in irradiated AIS preparations, indicating that irradiation induced extensive degradation of cell wall pectic polymers *in vitro*. Irradiated protein samples were affected by irradiation evident by the increased levels of total protein, and reduction in PG activity.

Irradiated mature-green fruit were more affected by irradiation than pink fruit. Effects of irradiation were in most cases dosage dependent. Pericarp tissue was more affected by irradiation compared to the whole fruit. Cell wall textural changes characterize the loss of firmness in mature-green fruit, while in pink fruit membrane alterations are partially involved.

CHAPTER 1
INTRODUCTION

The year 1895 marked the start of radiation biology when the German professor Wilhelm Conrad Roentgen discovered " X-rays " and in the following year (1896) Antoine Henri Becquerel, a French physicist, observed natural radioactivity In 1904 it was found that radiation has many biological effects, including the ability to inactivate bacteria, when Prescott found that radiation destroys spoilage bacteria in food However, there was no practical use of irradiation to treat significant food volumes until high-energy electron accelerators and nuclear reactors became available in the 1940s By 1966 safety studies on 21 foods had been conducted and the use of irradiation for some purposes such as inhibition of potato sprouting, disinfestation of wheat and its products, and sterilization of bacon and pork was approved by the US Food and Drug Administration (FDA) A world wide general standard was adopted in 1983 by the Codex Alimentarius Commission, which is responsible for world food standards By 1990 inspired by the Codex standard, 37 countries had approved one or more foods for irradiation The United States expanded its approval of the use of ionizing irradiation in 1986 to include fresh fruits and vegetables with a maximum dose of 1 kGy (100 Krad) in an effort to improve quality and reduce postharvest losses

Generally there are three forms of ionizing irradiation that can be used in food treatment gamma rays from sealed units of the radionuclides cobalt-60 or cesium-137 X-rays generated from machine sources at energies not exceeding 5 million electron volts, and electrons generated from machine sources at energies not exceeding 10 million electron volts Gamma rays collide with matter, causing ionization (e g removing electrons from the atoms or molecules) and, as a result, free radicals are produced consequently inducing some damaging effects upon the tissue The most common free radicals are those from water as most of the fresh commodities contain 85- 90% water and from oxygen in the air-filled space, which constitutes approximately 20% of the volume of some commodities (Pollard, 1983)

Ionizing radiation at various doses can be used depending on the tolerance of the commodity and the purpose of use, to prevent sprouting and growth, to control development and ripening, or to control insects and microorganisms (Couture et al 1990) In addition to the desired effects of irradiation, several undesirable effects such as increased pathogen susceptibility, physiological disorders, and changes in textural properties may occur Undesirable textural changes (softening) are perhaps the major problem repeatedly reported for irradiated fruits Effects of irradiation on textural properties have been reported for numerous fruits including pears, peaches, and nectarines (Maxie and Sommer, 1963), apples (Clegg et al , 1956 Boyle et al 1957 Phillips et al , 1960 Eric et al , 1970), lemons and oranges (Maxie and Sommer 1963), plums and raspberries (Truelsen 1963), grapes (Maxie et al , 1964) strawberries (Cooper and Salunkhe, 1963, Johnson et al., 1965 Brecht et al , 1992), sweet cherry (Massey et al ,

1965), and other organs including carrots (Skou, 1963). While Akamine and Moy (1983) concluded that eight out of 27 commodities benefit from irradiation in delaying ripening and senescence (banana, mango, papaya, sweet cherry, and apricot fruit), and suppressing rot organisms (tomato, strawberry and fig fruits), most of these commodities suffered from excessive softening as a result of irradiation. Irradiation-induced softening in tomato fruit has been reported to occur immediately following irradiation (Abdel-Kader et al., 1966c; Ahmed et al., 1972; Bramlage and Lipton, 1965; and Yasir et al., 1987). However, the range of techniques used in the above studies in preparing the fruit materials and/or in measuring firmness make it difficult to compare the results from these different studies. Although the adverse effects of irradiation on fruit texture have been known for many years, the precise mechanism of irradiation-induced texture loss is unknown.

The objective of the current study was to elucidate the mechanism of the textural changes associated with irradiation-induced softening of ripening tomato fruit. Particular emphasis was placed on examination of whole fruit and pericarp disc firmness loss over a period of time following irradiation. We also examined cell wall matrix polymers for possible modifications associated with irradiation-induced softening. This included measurement of total uronic acid content, polyuronide solubility and changes in molecular weight, pectin-associated neutral sugar composition, and hemicellulose molecular weight changes. Measurement of changes in cell wall-bound proteins and enzymes that might be altered by irradiation and/or altering the effect of irradiation on textural changes was also performed. Irradiation-induced membrane alterations and their

contribution to and/ or enhancement of the irradiation-induced softening were evaluated Tomato fruit AIS preparations and commercial pectin preparations were exposed to irradiation in order to study the direct impact of irradiation on polysaccharides and compare the *in vitro* changes to the observed *in vivo* alterations

CHAPTER 2
LITERATURE REVIEW

Introduction

Tomato (*Lycopersicon esculentum*) is a member of the Nightshade family (Solanaceae), which also includes capsicum pepper, eggplant, and Irish potato. It is believed that the modern tomato was developed from a primitive ancestor, the cherry tomato (*Lycopersicon cerasiforme*) originated first from the Peru-Ecuador area, and spread like a weed throughout Latin America. However, natives of Mexico were the first to domesticate the tomato plant due to its resemblance to the tomatillo (*Physalis ixocarpa*), which had long been used as a food. When the plant was first introduced to Europe by the Spanish, the Europeans recognized the plant as a member of the Nightshade family, which includes some plants containing poisonous substances, and thus considered it poisonous. The French, on the other hand, considered the tomato a stimulant to romantic desire, calling it the "love-apple" or "pomme d'amour". The tomato was brought back to the New World by the English colonists, however, it was not until the latter part of the nineteenth century that the tomato became a popular fruit in the United States.

The tomato fruit is a fleshy berry (swollen ovula in botanical terms) which is highly parenchymatous (Spurr, 1976). The fruit is mostly globular in shape, but

depending on the cultivar might also be oblate elongated or pear-shaped The body of the fruit, known as the pericarp is derived from the ovary wall that surrounds and encloses the seeds and the hypogynous ovary is syncarpous, consisting of two or more carpels The pericarp consists of outer (which might reach 1 cm in thickness) and radial walls (septa) that separate the adjacent carpels (Spurr, 1976) Major vascular bundles deviate from the base of the fruit (core) and intersect the pericarp and placenta with many other thin branches some reaching to the seeds (Spurr 1976) At an early stage of fruit development the placenta overgrows the seeds, partially disintegrating at maturity to form gelatinous material in the locules The structure of the skin is complex, consisting of relatively small, radially flattened cells (epidermis), and two to four layers of thick-walled collenchymatous cells (hypodermis) (Spurr 1976) The epidermis, that is lacking stomata, has a relatively thick external wall with the outer portion heavily cutinized, and an irregularly extended inward cutinization along the radial wall The outer surface of the fruit having a very thin layer of epicuticular wax, is relatively smooth.

The growth of tomato fruit follows a simple sigmoidal pattern and the first external color appearance on the fruit coincides with the completion of growth Cell division continues from anthesis until the fruit reaches 35% of its development Fruit harvested after this stage will ripen normally and will take approximately the same time to reach the breaker stage as the fruit left on the vine (Brady 1987)

Tomato is a typical climacteric fruit, which undergoes different metabolic changes associated with ripening in coordination with a climacteric rise in respiration These include a decrease in chlorophyll, an increase in carotene a conversion of starch to

reducing sugars an increase in ethylene production and other hydrocarbon volatiles, and an increase in tissue softening (Abeles, 1973)

The term "climacteric", which was first mentioned by Kidd and West (1933), is the sudden rise in respiratory rate and autocatalytic ethylene production (Abeles, 1973) associated with ripening, and marks the transition stage in the fruit development between maturation and senescence (Spencer, 1956) The climacteric period is defined as " The period in the development of some plant parts that involves a series of biochemical changes associated with the natural respiratory rise and autocatalytic production of ethylene" (Watada et al 1984 page 20) and consists of preclimacteric, preclimacteric minimum, climacteric rise, climacteric peak, and postclimacteric phases

The initiation of ripening in tomato fruit is marked by a rather large increase in ethylene production rates prior to the breaker stage of development of the fruit (Sawamura et al 1978, Su et al 1984, Brecht, 1985, 1986) and prior to the appearance of internal red color (Brecht, 1985, 1986) Chlorophylls a and b (that constitute the green color in unripe fruit) decrease rapidly during the transformation of chloroplasts to chromoplasts Lycopene and β-carotene are the two major pigments present in ripe tomato fruit, with a constant increase in the concentration of the former (the main red pigment) throughout ripening (Hobson and Davies 1971) Along with those mentioned above, changes in various acid and sugar components occur (Hobson, 1964)

Tissue softening is another major event that characterizes normal ripening of the fruit Softening of the fruit is believed to be a result of solubilization and degradation of pectic polysaccharides and loss of the cell wall neutral sugars galactose and arabinose

(Wallner and Bloom 1977, Gross and Wallner, 1979, Gross, 1984)

Pectinmethylesterase (PME) and polygalacturonase (PG) are considered the primary enzymes involved in softening However a considerable amount of evidence has been presented suggesting that PG is not solely responsible for cell wall structural changes occurring during normal ripening (Huber 1984 Seymour et al , 1987 Smith et al , 1988, Tucker and Grierson, 1982)

Plant Cell Wall

The structure of the plant cell wall mainly consists of two components microfibrils (a relatively rigid cellulose) and the matrix (non-cellulosic polysaccharides and glycoproteins) Chemically, the plant cell wall is generally composed of cellulose, hemicellulose pectins, and proteins.

Cellulose

Cellulose is a polysaccharide molecule with long unbranched chains of β-1,4 glycosidic-linked D glycopyranose residues A strong hydrogen bonding exists within the chain itself and among the different chains along their long axes The parallel arrangement of these molecules leads to the combined structural units called microfibrils (Frey-Wyssling, 1969) Rigidly held crystalline chains form the central core of the microfibril, whereas the amorphous portion can be found both in the central areas and on the microfibril periphery with less-aligned chains

Hemicellulose

Hemicellulose is made up of cell wall polysaccharides that include polymers of xylans, glucans glucomannans, galactomannans, arabinogalactans, callose and xyloglucans These polymers are insoluble in water or chelator but are soluble in strong alkali (Fry 1988), the property that characterizes this group (Towle and Whistler, 1973) Xyloglucan with a backbone composed of repeating cellobiose units similar to that of cellulose (Fry 1989, Hayashi 1989) is the most abundant hemicellulose polymer

Pectins

Pectin is composed of a regular structure of α-1,4-linked galacturonic acid units interrupted with rhamnose residues at specific areas of branching (BeMiller 1986 de Vries et al , 1986) The backbone of pectin is composed predominantly of long chains of galacturonic acid interrupted by rhamnose moieties to which are attached short chains of galactose and arabinose (Aspinall 1980 McNeil et al , 1984, John and Dey 1986) Galacturonic acid units in the main chain can be methyl-esterified at C-6 and also can be acetylated at C-2 and C-3 (McNeil et al , 1984)

Generally, three basic pectin classifications exist. Homogalacturonan (smooth' pectin) Rhamnogalacturonans I, Rhamnogalacturonan II

Homogalacturonan (smooth' pectin) Homogalacturonanas are unbranched and are methylesterified to variable degrees The backbone is composed of α-1 4-galcturonan residues that may be interrupted by rhamnose, introducing a conformational kink in the linear chain Regions that are not esterified hold a negative charge at the normal pH of the cell wall, giving them the ability to enter into ionic interactions with the calcium

present in the cell wall (Grant et al , 1973) The divalent calcium forms bridges, either between two charged regions in the same chain or between separate chains (Grant et al 1973)

Rhamnogalacturonan I This type of pectin contains in its backbone alternating galacturonic acid units and rhamnose units with side chains composed of arabinose and galactose (McNeil et al , 1984)

Rhamnogalacturonan II Rhamnogalacturonan II contains in its backbone a linear chain of α-1,4-galacturonic acid with side chains of rhamnose, galactose, arabinose, fucose, glucuronic acid, 2-o-methylfucose, 2-o-methyxylose, apiose, acetic acid, 3 deoxy-D-manno-2-octulosonic acid and 3-deoxy-D-lyxo-2-heptulsaric acid (Darvill et al 1978 Stevenson et al , 1988) Even though this type of pectin is a relatively small polymer, it is considered to be one of the most complex polysaccharides known

Proteins

Proteins are found either as structural or enzymic components of the cell wall There are three identified structural proteins in the cell wall These include hydroxyproline-rich glycoproteins (extensin) (Cassab and Varner 1988), glycine-rich proteins (Keller et al 1988) and arabinoglactan proteins (Fincher et al , 1983) Several enzymes have been identified in protein isolates prepared from isolated cell walls from a variety of fruits Those include exoPG and β-galactosidase in apple fruit (Bartley 1974,1977 1978), α-, and β-galactosidases and glucosidases, α-mannosidase α-arabinosidase, β-xylosidase, PME and PG in pear fruit (Ahmed and Labavich 1980, Bartley et al , 1982), β-galactosidase, β-1,3-and exo-β-1,4-glucanase, C_x-cellulase, PME,

enzymes have been identified in protein isolates prepared from isolated cell walls from a variety of fruits. Those include exoPG and β-galactosidase in apple fruit (Bartley, 1974,1977 1978), α- and β-galactosidases and glucosidases, α-mannosidase, α-arabinosidase β-xylosidase PME and PG in pear fruit (Ahmed and Labavich, 1980, Bartley et al 1982), β-galactosidase, β-1,3-and exo-β-1 4-glucanase C_x-cellulase, PME and endo PG in tomato fruit (Kivilaan et al 1961, Sobotka and Stelzig, 1974, Buescher and Tigchelaar, 1975, Strand et al , 1976, Wallner and Bloom, 1977, Poovaiah and Nukaya, 1979, Gross and Wallner, 1979 Tucker et al , 1980 Themmen et al , 1982 Hubur and Lee, 1988)

Irradiation

Generally, there are three forms of ionizing irradiation that can be used in food treatment gamma rays from sealed units of the radionuclides cobalt-60 or cesium-137 X-rays generated from machine sources at energies not exceeding 5 million electron volts and electrons generated from machine sources at energies not exceeding 10 million electron volts Gamma rays, the form that is used most often, collide with matter causing ionization (i e removal of electrons from the atoms or molecules) and as a result of this ionizing nature of gamma rays, free radicals are produced, consequently inducing some damaging effects upon the tissue The most common free radicals are those from water as most of the fresh commodities contain 85-90% water and from oxygen in the air-filled space which constitutes approximately 20% of the volume of some commodities (Pollard, 1983) Ionizing radiation at various doses can be used, depending on the

of commodity and cultivar production area and season, maturity at harvest, initial quality and type of postharvest procedures as well as factors related to irradiation procedures such as dose of irradiation, dose rate, and conditions surrounding the produce during irradiation such as temperature and atmospheric composition (Kader, 1986)

General Effects of Ionizing Irradiation at Various Doses on Fresh Fruits and Vegetables

Effect on respiration and ethylene production rates The effect of irradiation on respiration and ethylene production rates for many fruits and vegetables has been studied by several workers, who concluded that irradiation generally enhances respiration and ethylene production rates For example an increase in respiratory rate was observed in lemons irradiated at 0 25-1 0 kGy (Maxie et al 1965) Increased respiration and ethylene production rates proportional to the irradiation dose have been reported in strawberry fruit irradiated at 0 3 1 0, 2 0, or 3 0 kGy (Couture et al 1990) In mature-green tomato fruit, Burns and Desrosier (1957) reported an increases in respiration rates immediately following irradiation at doses of 0 5, 1 0 or 1 5 kGy and that the increase was a function of the irradiation dose used In addition, irradiation of mature-green, pink, and table ripe tomato fruit at irradiation doses from 0 5 -7 0 kGy caused a dosage dependent increase in the respiratory rate (Abdel-Kader et al 1968a) Ethylene production, however decreased at doses above 6 0 kGy but was stimulated below 5 0 kGy, although the increase in ethylene production was not accompanied by an increase in ripening rates in these tomato fruit (Abdel-Kader et al 1968a)

Generally irradiation with doses above 1 0 kGy enhanced the respiration rates of both climacteric (pear peach) and non-climacteric (strawberry, cherry) fruits It is believed, though, that higher doses of irradiation cause a reduction in ethylene sensitivity of most fruits (Maxie and Abdel-Kader 1966)

Inhibition of sprouting and postharvest growth Sprouting of tubers, bulbs and root vegetables (potato yam, Jerusalem artichoke, sweet potato ginger sugar beet table beet, turnip carrot, onion, and garlic) can be inhibited at doses between 0 05-0 15 kGy and doses below 0 15 kGy have only minor effects on the quality of these commodities (Kader, 1986) Such doses can also be used to prevent elongation and curvature in asparagus spears, but higher doses tend to have detrimental effects on the quality and storage life of the spears (Kader, 1986) Irradiation doses of 0 06-0 50 kGy are required to inhibit postharvest growth of mushrooms, not only preventing cap opening and stalk elongation, but also reducing surface molds and darkening of the gills and preserving the fresh appearance of the mushrooms (Kader 1986)

Insect disinfestation. It has been postulated that the use of ionizing irradiation for insect disinfestation is one of the most promising applications (Burditt, 1982 1983 Moy et al 1983 Moy, 1985) Doses below 1 0 kGy are in most cases an effective insect disinfestation treatment against various species of fruit flies, mango seed weevil, oaval orange worm, potato tuber moth, codling moth, spider mites, scale insects and other insect species (Kader 1986) Sterilization of most insects can be achieved at doses of 0 05-0 75 kGy For example, it was found that the minimum mortality dose for mature Medfly eggs in peach fruit was 0 04 kGy, while in nectarines it was between 0 45-0 50

kGy (Moy et al 1983) Generally eggs are most sensitive to irradiation followed by larvae, then pupae. Most fresh fruits and vegetables can tolerate an irradiation dose of 0 25 kGy with minor effects on quality, however, damage could occur between 0 25 and 1 00 kGy (Kader 1986)

Physiological disorders A common form of tissue death caused by irradiation is the pitting of citrus fruit Hatton et al (1984) reported an increase in rind breakdown, mainly pitting, induced by irradiation in 'Ruby Red' and 'Marsh grapefruit. However they suggested that the increased pitting was not serious enough to pose serious marketing problems unless the dose exceeded 0 60 kGy (Hatton et al , 1984) Doses of ionizing irradiation above 1 0 kGy may also cause various types of physiological disorders in fresh fruits and vegetables (Bramlage and Couey, 1965 Bramlage and Lipton, 1965, Lipton et al 1967 Maxie et al 1971) Such physiological disorders include increased surface blemishes, swelling of the oil glands, and peel pitting in oranges and grapefruit, internal cavities in lemons and limes skin damage in banana, internal browning of avocado, skin discoloration and stem darkening in grapes, internal and external discoloration of olives, enhanced yellowing of cucumbers summer squash and pepper stem pitting in artichoke, and enhanced sensitivity to chilling injury in several crops (Kader 1986)

Nutritional quality. The nutritional quality or caloric values of fresh fruits and vegetables are not significantly altered by irradiation at doses below 3 0 kGy (Kader 1986) The loss of niacin, thiamin, riboflavin and beta carotene (provitamin A) in irradiated fruits and vegetables is insignificant (Kader, 1986) However, 0 to 95% losses

in ascorbic acid may occur as a result of ionizing irradiation due to its radiosensitivity which depends on the commodity, cultivar, dose of irradiation and duration and temperature of storage (Maxie and Abdel-Kader, 1966). Pigments, sugars, fats, proteins and enzymes have all been reported to be subject to slight changes in most cases when fruits and vegetables are irradiated with doses below 3.0 kGy. Some other compositional changes occur due to irradiation that might be desirable, such as the decrease in acidity in some commodities, loss of astringency in persimmons, increased juice yield in grapes and inhibition of chlorophyll and solanine formation in potato tubers exposed to light. Unpleasant flavors and aromas can be detected after irradiation of some commodities with doses above 1.0 kGy, which can be intensified with time after exposure to irradiation (Sommer and Mitchell, 1986). Nevertheless, Akamine and Moy (1983) reported that a dose of only 0.2 kGy negatively affected the flavor and aroma of 'Dancy' tangerines. Matsuyama and Umeda (1983) observed that potatoes irradiated to prevent sprouting became sweeter and darker upon cooking compared to non-irradiated controls.

Control of postharvest disease. Control of postharvest diseases by the use of ionizing irradiation depends on the sensitivity of pathogens to irradiation relative to the tolerance of the host (Kader, 1986). In general, the minimum dose required for effective control of fungal pathogens is 1.75 kGy; however, a dose of 2.25 kGy has been reported to be to the maximum dose that does not inflict serious effects such as loss of firmness, abnormal ripening, off-flavor and increased susceptibility to mechanical injury in most commodities (Sommer and Fortlage, 1966). Salunkhe (1961) observed that both the quality and the mold population of irradiated green, pink and ripe tomato fruit were

reduced when the irradiation dose was increased above 1 0 kGy During 14 days storage the deterioration of irradiated pink tomato fruit was slower than irradiated red fruit (Salunkhe, 1961) Mathur (1963) reported that decay of green tomato fruit irradiated at doses of 0 5-0 8 kGy and held at room temperature following irradiation was slower than that of the conti ol fruit Truelsen (1963) reported that doses of 0 5, 1 0, 2 5, and 5 0 kGy were not effective in delaying rot or preserving the taste and texture of tomato fruit in storage. Burns (1956) reported that mature-green tomatoes irradiated with 10 0 kGy and then inoculated with *Erwinia aroidae* deteriorated more rapidly than non-irradiated inoculated controls and suggested that radiolytic depolymerization of pectins was the reason for such deterioration Proctor et al (1953) found that mold growth on tomato fruit was inversely related to doses between 0 093 and 0 465 kGy Beraha et al , (1960) found that for the young, actively growing mycelia of *Alternaria tenuis* Auct on tomato fruit, the lethal dose of gamma radiation was between 2 75 and 4 56 kGy, without mentioning the size of the fungus population, which led him to conclude that *Alternaria* spp are rather resistant to irradiation Mature-green and breaker tomato fruit irradiated at 1 0- 3 0 kGy exhibited a decrease in shelf life due to increased decay (Abdel-Kader et al 1968b) They suggested that the increased susceptibility of the fruit to infection could be due to the irradiation-induced softening of the fruit, which is in agreement with the previous reports by Burns (1956) and Truelsen (1963) It has been observed that irradiation at doses below 1 0 kGy enhanced the susceptibility of some commodities to rotting microorganisms. For example topped carrots irradiated with 0 12 kGy to prevent sprouting exhibited 4-5 times more rotting caused by *Botrytis cinerea* Pers. ex Fr and

Sclerotinia sclerotiorum (Lib) than control carrots after 5 5 months storage at 0 and 2°C (Skou 1963) Foa et al (1980) reported that irradiation of Spadona pears with 0 50 kGy resulted in scald-like symptoms of skin browning, browning of the calyx, and some skin cracking It was found that doses below 2 5 kGy used to irradiate tomato fruit at the pink stage were not effective in reducing decay during holding for 20 days at 20°C whereas doses above 2 5 kGy were effective in reducing the amount of rot, with 3 0 kGy the most effective dose in this case (Abdel-Kader et al , 1968b)

Irradiation-Induced Softening

Undesirable textural changes (loss of firmness or excessive softening) are perhaps the major problems repeatedly reported for irradiated fruits, including tomatoes (Abdel-Kader et al 1968c) Exposure of plant tissue to ionizing irradiation can result in changes in texture or firmness (Brasch and Huber, 1947, Morgan, 1955 Hannan, 1955) Softening of fruits caused by ionizing irradiation has been reported in pear, peach and nectarine (Maxie and Sommer 1963), in apple (Glegg et al , 1956 Boyle et al , 1957, Phillips et al 1960) lemon and orange (Maxie and Sommer, 1963), plum and raspberry (Truelsen 1963), grape (Maxie et al , 1964) strawberry (Cooper and Salunkhe 1963, Johnson et al 1965 Brecht et al 1992), sweet cherry (Massey et al 1965), and tomato (Abdel-Kader et al , 1968c Bramlage and Lipton 1965 Ahmed et al., 1972 Yaur et al , 1987) fruits The threshold dose that may cause such changes varies significantly for various plant tissues (Glegg et al 1956 Boyle et al., 1957) Peach fruit irradiated at doses of 0 3-0 9 kGy exhibited an immediate softening but by the fourth day there was no difference between treated and control fruit (Somogyi and Romani, 1964) Strawberry

fruit irradiated with 1 0 and 2 0 kGy suffered from softening leaking, and bleaching of external color (Johnson et al , 1965) A dose of 3 0 kGy caused the berries to suffer from a spongy water-soaked texture (Johnson et al. 1965) Strawberry fruit subjected to a shear-press test 1 6 and 10 days after irradiation with 0 2 0 or 4 0 kGy required less crushing force with increased doses of irradiation, indicating progressive softening with increased irradiation (Johnson et al , 1965)

Firmness of tomato fruit irradiated at mature-green, breaker pink, and table ripe stages was lost proportionally to dose immediately after irradiation, but the effect decreased after ripening of the fruits (Abdel-Kader et al., 1968c) Tomato fruit irradiated at 1 25, 2 50 or 5 0 kGy were significantly softer as measured subjectively using a rating scale compared to non-irradiated fruit (Bramlage and Lipton 1965) It was found that the riper the fruit at the time of irradiation, the softer it became (Bramlage and Lipton, 1965) Firmness of tomato fruit as determined by sensory evaluation decreased as a function of increasing dose of irradiation, and tomato fruit softened progressively with longer storage periods (Ahmed et al , 1972) Softening of irradiated tomato fruits was more pronounced in the first 5 days of storage than after 7 days (Ahmed et al 1972) Abdel-Kader et al (1968c) also reported that firmness of irradiated tomato fruit determined by Kramer Shear Press decreased proportionally to the radiation dose immediately following irradiation, however, as the fruit ripened the effect decreased

Irradiation Effects on Cell Wall Pectic Polysaccharides

Considerable research has been directed toward understanding the mechanism of the textural changes associated with normal ripening of fruits, which include

solubilization and degradation of pectic polysaccharides and loss of the cell wall neutral sugars galactose and arabinose in tomato fruit (Wallner and Bloom, 1977, Gross and Wallner 1979, Gross, 1984) and many other fruits (Labavitch, 1981) Similarly solubilization of pectic polysaccharides and the release of uronic acid (Kertesz et al 1956 McArdle and Nehemias, 1956 Boyle et al , 1957 Kertesz et al , 1964, Somogyi and Romani, 1964, Yasir et al., 1987 d'Amour et al , 1993) associated with the hydrolysis of polysaccharides to lower molecular weight fragments (Maxie and Abdel-Kader 1966) have been considered the major events that characterize irradiation-induced softening

A common approach to studying the changes in the different soluble pectic fractions in irradiated fresh fruits and vegetables in the 1950s and 1960s was the sequential extraction with water (for pectins), followed by ammonium oxalate (for pectates), and finally with hydrochloric acid (for protopectins) The total amount of pectic substances was considered to be the sum of the three extracted fractions on a weight basis Based on such analysis, decreases in total pectic substances were reported in carrot roots and apple fruit irradiated at 0 5-2 0 kGy (McArdle and Nehemias 1956) and in pear and peach fruits irradiated at 3 0, 6 0 or 9 0 kGy (Somogyi and Romani 1964) Breakdown of water- and ammonium oxalate-soluble pectic fractions to simpler non-pectic materials occurred in carrot and apple, along with total destruction of the hydrochloric acid-soluble fraction at the highest dose (2 0 kGy), which is presumably the fraction responsible for the loss of tissue firmness noted in those two crops (McArdle and Nehemias 1956)

Somogyi and Romani (1964) reported a significant decrease in the hydrochloric acid-soluble fractions (as a function of dose), a two-fold increase in the water-soluble fractions and a slight increase in the ammonium oxalate-soluble fractions in irradiated pear and peach fruits. Although a sharp decrease in the hydrochloric acid-soluble fraction in irradiated pear fruit was noted immediately following irradiation, the values became higher than those of the control after holding for 4 days at 20°C during which time the control fruit eventually became softer than the irradiated fruit. It was concluded that the more pronounced textural changes observed in the control pear fruit concurred with the solubilization of the hydrochloric acid-soluble fraction (Somogyi and Romani 1964).

On the other hand, in two groups of strawberry fruit irradiated at 2.0 kGy no change was noted in total pectic substances in the first, while an increase was noted in the second group relative to the controls (Belli-Donini and Stornaiuolo 1969). The different behavior of the two strawberry groups (Belli-Donini and Stornaiuolo 1969) was attributed to the presumed correlation between changes in pectins and calcium ions, changes in pH of the cell wall and changes in cell permeability during ripening of the fruits (Doesburg, 1957; Esau et al., 1962). Furthermore, a slight increase in the hydrochloric acid-soluble fraction was noted in the first days of storage at 0-3°C after which a dramatic decrease was noted, and the values in irradiated fruit were always lower than the control in both experiments. The ammonium oxalate-soluble pectic fraction showed a sharp increase but the control was noted to be higher for the last 2 weeks in one group of strawberry fruit whereas no differences were observed between control and irradiated fruit in the second group (Belli Donini and Stornaiuolo 1969). The water-

soluble pectic fraction in irradiated fruit was higher than the control in both groups (Belli-Donini and Stornaiuolo, 1969). Since a smaller content of oxalate-soluble pectic substances was noted, and no effect on PME activity was detected, it was concluded that a possible correlation existed between the enzymic activity and the oxalate-soluble pectic substances content in the irradiated fruit (Belli-Donini and Stornaiuolo 1969). The decrease in the extractable oxalate-soluble fraction could also be due to modification of the ionic equilibrium of the internal cell sap as a consequence of modified cell wall permeability which would then result in a reduction of the available calcium, leading to a reduction in the amount of oxalate-soluble pectic fraction (Belli-Donini and Stornaiuolo 1969). Irradiation of riper berries had resulted in an increase in the water soluble fraction content, probably due to the irradiation effect on the hydrochloric acid-soluble fraction, which is bound to cellulose and hemicellulose, either by releasing it from the cell wall or degrading (converting) it to a water-soluble fraction (Belli-Donini and Stornaiuolo 1969).

It was found that in grapefruit and orange fruits irradiated at 1.5-3.0 kGy, the water-, and oxalate-soluble pectic fractions increased relative to the control, but the sodium hydroxide-soluble fraction (protopectin) was noted to decrease (Rouse and Dennison 1968). In their conclusion, Rouse and Dennison (1968) referred to similar results reported previously (Kertesz et al., 1956), and suggested that the more mature the fruit at the time of irradiation the less the effect of irradiation on pectin grade (a factor used to determine the ability of pectin to form jelly). They noted that sugars added to pectin solutions before irradiation were known to protect the latter from irradiation (Kertesz et al., 1956), and stated that since the total sugars increase during maturation of

grapefruit and orange fruits (Ting and Deszyck 1961) consequently the increased sugars might have protected pectins from damage by irradiation at an advanced maturity stage (Rouse and Dennison, 1968)

More recent studies have employed more advanced methods in the analysis of the modifications that occur in cell wall components in fruits and vegetables exposed to ionizing irradiation Using the pectin extraction procedures of Ahmed and Labavitch (1977) and the uronic acid determination assay method of Blumenkrantz and Asboe-Hansen (1973) as modified by Kintner and Van Buren (1982), Howard and Buescher (1989) reported that the solubility properties of pectic substances in irradiated cucumber pickles were altered by increasing the dose of irradiation According to these authors, the total pectic substances (measured by uronic acid assay) were unaffected in AIS derived from pickling cucumber irradiated at 0 5 or 1 0 kGy However, irradiation with 1 0 kGy resulted in reduced levels of the sodium hydroxide soluble and non-extractable fractions, and increased levels of water-soluble pectins and sodium hexametaphosphate-soluble pectin (Howard and Buescher 1989) The non-extractable pectins have been reported to play a significant part in maintaining the firmness of cucumber pickles (Hudson and Buescher 1985) Breakdown of this portion by PG resulted in an increase in the sodium hexametaphosphate-soluble portion and reduced firmness during storage of cucumber pickles (Howard and Buescher 1989) It is presumed that demethylation and modification of cell wall structure in response to irradiation resulted in greater accessability of free calcium ions to negatively charged carboxyl groups on pectin molecules (Howard and Buescher, 1989) and that the resulting formation of calcium

pectates increased the rigidity and cohesive properties of structural components (Van Buren, 1979)

In strawberry fruit a decrease in galacturonate from 59% to 52% of the total cell wall carbohydrates was noted in response to irradiation at 4 0 kGy (d Amour et al , 1993) A large decrease in water-insoluble pectins was noted in irradiated strawberry fruit, while there was no change in water-soluble pectin, indicating that degradation of pectin chains into more soluble fractions with lower molecular weight had occurred (d Amour et al 1993) It is believed that galacturonic acid, that was reduced mainly from the water-insoluble pectins and slightly from hemicellulose fractions, was degraded to shorter chains of water-soluble pectin, whereas an equal amount of pectic chains are believed to be lost as short ethanol-soluble chains which might account for the observed lack of change in water-soluble pectins (d Amour et al , 1993)

Yasir et al (1987) found that the total pectic substances (sum of the three soluble fractions) was unaffected in mature-green tomato fruit irradiated at 0 5 to 5 0 kGy No change also was noted in the water-soluble fractions, while an increase was noted in the sodium hexametaphosphate-soluble faction that compensated for the decrease noted in the hydrochloric acid-soluble fractiou (Yasir et al , 1987) In their conclusion, Yasir et al (1987) stated that the conversion (i e. degradation) of the hydrochloric acid-soluble (protopectin) fraction to either water- or sodium hexametaphosphate-soluble (pectate) fractions was the reason behind tissue softening in the irradiated fruit

Pectin degradation has also been measured by the change in pectin viscosity and molecular weight at doses as low as 0 4 kGy (Sommer and Mitchell, 1986) Using *in*

vitro models, depolymerization was observed in irradiated solutions of pectin (Skinner and Kertesz, 1960 Kertesz et al , 1956) and cellulose (Glegg and Kertesz, 1957) The loss of viscosity as a measure of the degradation of pectins in irradiated fruits and vegetables, is thought to indicate both depolymerization of pectins and changes in pectin fractions (McArdle and Nehemias 1965, Kertesz et al , 1964) Upon treatment of aqueous pectin solutions or dry pectins with ionizing irradiation at various doses, degradation of pectins was evident by decreases in solution viscosity of irradiated pectin solutions (Kertesz et al 1956 Dzamic et al , 1966), and irradiated dry pectin (Kertesz et al , 1956, Glegg et al , 1956) Other investigators (McArdle and Nehemias, 1956, Rogachev, 1966 Maxie et al 1968 Massey 1968) reported that irradiation caused decreases in viscosity and total pectic substances with increasing exposure time and a breakdown of the hydrochloric acid-soluble fraction (protopectins) to ammonium oxalate-(pectates) and water-soluble fractions (pectins)

In previous studies of the solubility and M_v changes in specific pectic fractions in response to irradiation of fruits (Somogyi and Romani, 1964, d'Amour et al 1993), the procedures for isolation of pectic polysaccharides involved exposure to potentially degradative treatments (high temperatures and/ or acid) In other cases (Yasir et al 1987) there was no indication that attempts were made to inactivate endogenous pectin-depolymerizing enzymes Consequently, it is difficult to attribute the observed changes in pectin properties to the effects of irradiation versus thermal or enzymic degradation unrelated to the irradiation treatments

Depolymerization of pectic substances results from free radicals, which are common radiolytic products when materials in aqueous media are exposed to gamma irradiation (Urbain, 1986). Although degradation of one fragment influences the cohesiveness of the cell wall and the extractability of others (Goodman et al. 1986), it is difficult to explain the direct effect of irradiation on a particular fraction (d'Amour et al., 1993). It was found in a preliminary experiment that softening of pear, peach and nectarine fruits was much less when irradiation treatment occurred under nitrogen atmosphere than under normal air (Somogyi and Romani, 1964). Apparently the oxygen-free atmosphere caused reduction in the formation of free radicals. However, irradiation of 1% solutions of pectins under either normal air and/or nitrogen showed no response to the presence or absence of oxygen, although viscosity changes were observed (Somogyi and Romani, 1964). This may suggest that the radio-chemical changes evident by the reduced viscosity in pectin could be a direct effect of irradiation rather than mediated by oxidizing radicals (Somogyi and Romani, 1964). Cell wall damage by random breaks in glycosidic linkages of pectins (Skinner and Kertesz, 1960) and degradation of other cell wall components (Foa et al. 1980) as a result of irradiation probably contribute to the softening of fruits and vegetables (Sommer and Mitchell, 1986).

Irradiation Effects on Cellulose and Hemicellulose

The role of cellulose and hemicellulose degradation in the textural changes that occur following irradiation of fresh fruits and vegetables is not clear because little attention has been given to studying these cell wall structural components.

Irradiation of cotton and wood cellulose at 0 01-2 21 kGy was conducted *in vitro* at different moisture contents (Glegg and Kertesz, 1957) Results indicated that irradiation caused a decrease so cellulose viscosity immediately following irradiation, and longer holding in solution did not affect the viscosity further Furthermore, there was no effect of moisture content on the degree of degradation measured by the decrease in viscosity When irradiated samples were dried and held for 35 days, periodic measurements indicated a decrease in viscosity until 30-35 days following irradiation It was found that irradiation doses that caused degradation of cellulose were either equal to or below the threshold irradiation doses that induced softening in apples and carrots (Boyle et al , 1957), and in beets (Glegg et al , 1956) which led the authors to conclude that cellulose degradation could be a factor in the softening However, the authors assumed that cellulose has to be degraded substantially before any cell wall structure can be weakened and measurable softening can be produced (Glegg and Kertesz, 1957)

In strawberry fruit irradiated at 4 0 kGy, cellulose analysis by colorimetric determination of glucose revealed low glucose levels probably because of incomplete hydrolysis, and the galacturonic acid content of cellulose gave some values that indicated some contamination with pectic substances (d' Amour et al , 1993) It has been concluded that the reduction in galacturonic acid content was reduced mainly from water-insoluble pectic fractions and slightly from cellulose and hemicellulose fractions Furthermore, the water-insoluble pectic fraction and hemicellulose fractions were degraded to shorter chains of water-soluble material, while an equal amount was lost as ethanol-soluble chains from the water-soluble pectin fraction (d'Amour et al 1993)

Irradiation Effects on Pectin-Associated Neutral Sugars

It is well established that during normal ripening of many fruits the levels of arabinose and galactose associated with pectic polymers (Darvill and McNeil, 1980) decrease during ripening (Knee 1975, Yamaki, 1979 Gross and Wallner 1979) In tomato fruit, 65% of the cell wall galactosyl residues are lost during normal ripening (Gross, 1984 Gross and Wallner, 1979, Lackey et al , 1980), a process that is believed to be independent of pectin degradation (Bartley, 1976, Gross and Wallner 1979 Knee, 1973) While a reduction in the pectic substances was observed in refrigerated cucumber pickles irradiated at 1 0 kGy, the level of total neutral sugars was only slightly affected However there was a significant reduction in galactose and no significant changes were observed in rhamnose, arabinose, fucose, mannose, xylose and glucose or in the total neutral sugars (Howard and Buescher 1989) Neither was any effect on total neutral sugars noted in irradiated strawberry (d'Amour et al. 1993) Galacturonic acid and glucose were the two components of the cucumber cell wall most affected by gamma irradiation, and the increase in glucose suggested that cellulose is hydrolyzed following irradiation (d'Amour et al , 1993) Galcturonic acid as a portion of the total cell wall carbohydrates decreased by 7%, which suggests depolymerization of the polygalacturonic acid chains but not the neutral sugar side chains, as the neutral sugars were only slightly affected compared to the galacturonic acid (d Amour et al 1993) It was suggested that the embedded neutral sugar side chains of the branched pectic substances in the cellulose and hemicellulose network might be less susceptible to depolymerization by the free radicals during gamma irradiation (d'Amour et al , 1993) The role of the neutral sugars

in irradiation-induced softening is still unclear Therefore it was concluded that pectin solubilization is probably the principal contributor to the softening induced by gamma irradiation (Howard and Buescher 1989, d'Amour et al 1993)

Irradiation Effects on Shelf Life and Quality

The ability of ionizing irradiation to extend the shelf life of fresh fruits and vegetables has been investigated extensively for the past 50 years or so However, large differences in the reports dealing with the estimation of shelf life extension of horticultural commodities exist (Sommer and Mitchell 1986), including the effect of irradiation on shelf life and quality of tomato fruit (Abdel-Kader et al , 1968b) For example, some investigators have reported an extension of 2-5 days in the shelf life of strawberry fruit irradiated at 2 0 kGy and held at 0°C, while others have estimated the extension to be up to 30 days under similar conditions (Sommer and Mitchell, 1986) In tomato fruit, Proctor et al (1953) reported that pink and full red fruit irradiated at 2 0 3 0 or 4 0 kGy were more acceptable than the non-irradiated fruits Brunnelet and Vidal (1960) reported that green tomato fruit irradiated at a dose of 3 0 kGy could be held one month longer than non-irradiated controls In other cases, the shelf life was reported to be significantly shortened by irradiation (Bramlage and Couey 1965, Lipton et al 1967 Maxie et al 1971) In general, treatment with irradiation at doses of 1 0-3 0 kGy which might be used to control some microorganisms, resulted in only a very short (2-5 days) shelf life extension (Sommer and Mitchell 1986) Such observations were explained by irradiation-induced softening, which counters the beneficial effect of irradiation in suppressing microorganisms (Sommer and Mitchell, 1986) Increases in shelf life of 3,

13, 9 and 6 days were reported in pink tomato fruit irradiated at 3 0 kGy and held at 15 20, 25, and 30°C respectively (Abdel-Kader et al , 1968b) The shelf life of tomato fruit irradiated at both mature-green and breaker stages of development was found to be extended 1-3 days at an irradiation dose of 4 0 kGy while a reduction of 1-2 days was noted at 1 0 2 0 and 3 0 kGy (Abdel- Kader et al , 1968b) A great reduction in shelf life was noted at irradiation doses of 5 0-7 0 kGy, which had a severe negative effect on the quality of the tomato fruit (Abdel-Kader et al 1968b) The shelf life of 'table-ripe' stage tomato fruit was extended when irradiated at different doses An irradiation dose of 3 0 kGy was noted to be the most effective as it increased the shelf life by 10 days (Abdel-Kader et al , 1968b)

Irradiation Effects on Fruit Ripening and Color Development

Research regarding the effect of irradiation on ripening and color development has led to diverse conclusions for different fruits with a wide range of results that have been reported indicating both delay and acceleration of ripening (Sommer and Mitchell 1986) Inhibition of ripening has been reported for mango (Hatton et al , 1961), papaya and pears (Maxie and Sommer, 1963), banana (Amezquita, 1965 Hannan 1956) guava and some other subtropical and tropical fruits (Akamine and Moy 1983 Thomas, 1986) A dose as low as 0 3 kGy has been reported to delay ripening of some tropical fruits such as papaya, mango and banana, whereas temperate-zone fruits such as apple pear and apricot require doses above 1 0 kGy for effective inhibition of ripening (Kader 1986)

Ripening of Bartlett pear fruit has been reported to be delayed for several days after irradiation at a dose of 1 0 kGy, but with an abnormal ripening and dry and mealy

flesh (Bramlage and Couey 1965) At higher doses pear fruit retained an abnormal green mottling on the surface, and were not able to develop the characteristic flavor of ripe fruit, even after treatment with ethylene gas (Bramlage and Couey, 1965, Maxie and Abdel-Kader 1966) However, Maxie and Abdel-Kader (1966) reported early ripening of 'Elberta' and 'Sun Crest' peaches after their exposure to 1 0 kGy of irradiation. The retardation of ripening in irradiated tomato fruit increased with increasing dose and decreased with the advance of maturity stage at the time of irradiation (Abdel-Kader et al 1968b)

It was found that irradiation of pineapple fruit at doses of 0 3-0 5 kGy delayed degreening (Upadhya and Brawbaker 1966) whereas Maxie et al (1965) reported that irradiation of 'Eureka' lemons with doses of 0 25 to 1 0 kGy hastened degreening as a result of irradiation-induced ethylene production. The change in ground color from green to yellow in 'Red Globe' and 'Halloween' peaches and 'Late Le Grand' nectarines was hastened when these fruits were exposed to irradiation at 0 45 kGy , whereas such a dramatic change was not observed in 'Elberta' peaches (Bramlage and Couey, 1965)

Irradiation has been reported to enhance the yellow color in lemons, and yellow and red color in peaches (Maxie et al 1964) whereas irradiation of strawberry which is a non-climacteric fruit, at 0 30 kGy might delay color development when used with 10% CO_2 to control microorganisms (Couture and Willemot, 1989), and strawberry fruit irradiated at 1 0 kGy showed less intense color when stored for 1 week at 7° C or 2 weeks at 1°C (Brecht et al , 1992)

Herregods (1963) reported that red color development of tomato fruit was delayed during holding at 15°C following irradiation at 4 0 kGy However, Burns and Desrosier (1957) reported that an increase in red pigmentation occurred in tomato fruit at higher irradiation dosages for some time during the experiment and suggested that this might be related to the availability of the substrate utilized in the enzymatic processes involved, which might have been enhanced by the increase in respiratory activity (Burns and Desrosier 1957) Nevertheless, a final inhibition of the productioo of red color was observed in tomato fruit treated with ionizing irradiation (Burns and Desrosier 1957) Tbese results showed that the inhibition of the normal ripening-related red color development was dne to the inhibition of synthesis of gamma carotene and lycopene judged by the decreases in these pigments, and that fruit maturity at the time of irradiation had oo significant effect on the response to irradiatioo in terms of color development except for gamma-carotene, whereas beta-carotene levels were not affected by treatment with ionizing irradiation (Burns and Desrosier 1957) Oo the other hand, irradiation with 1 25 and 2 50 kGy prevented red color development of mature-green 'Early- Pack' tomato fruit in one ont of two tests (Bramlage and Lipton, 1965) irradiation at 3 75 kGy delayed coloring of 'Pearson' tomato fruit, but they eventually attained full red color (Bramlage and Lipton, 1965), whereas 5 0 kGy prevented the fruit from attaining full red color aven when held beyond the normal ripening period (Bramlage and Lipton 1965) The effect of irradiation also was reported to be a function of the stage of the development of the fruit, as the inhibition of color development was more pronounced in irradiated mature-green fruit than in irradiated breaker or pink fruit (Bramlage and Lipton

1965 Abdel-Kader et al 1968c) In addition, irradiation also affected the uniformity of coloration, as the irradiated tomatoes retained green or yellow areas in the overall pink or red colored fruit (mottling) (Bramlage and Lipton, 1965) Green tomato fruit irradiated at 1 0 kGy exhibited uneven ripening and failed to develop red color (Rivers 1959 Salunke, 1961) It has been reported that color development was delayed for 4-8 days at 20°C in mature-green tomato fruit irradiated at 4 0 kGy and 1-2 days with lower doses (Abdel- Kader et al 1968a) Irradiation with 3 0 kGy and above caused uneven coloration (irregular mixture of green, yellow and red color) of tomato, and the number of fruit with uneven coloration increased with increasing irradiation dose (Abdel-Kader et al 1968a) Irradiation at 4 0 kGy caused a delay of color development when the fruit held for only 2 days at 25°C, however, none of the fruit reached a desirable red color (Abdel-Kader et al , 1968a) In other experiments doses of 3 0 kGy and below had no significant effect on color development of mature green fruit when held at 20°C (Abdel-Kader et al 1968a) Even though doses of 4 0 and 5 0 kGy caused a delay in color development, all the fruit reached full color whereas fruit irradiated at 6 0 and 7 0 kGy failed to develop the red color typical of ripe tomato fruit, and it was concluded that 4 0 kGy was the minimum dose for inhibition of ripening (Abdel-Kader et al , 1968a)

Irradiation Effects on Membranes

The composition of polar lipids and the permeability of membranes in plants are influenced by various types of stress such as dehydration (Bewley 1979, De Paula et al 1990 Robinson, 1966 Stewart and Bewley, 1982), osmotic stress (Zwiazek and Blake 1990), low temperature (Clarkson et al , 1980; Ferks et al , 1975, Ketchie and Kuiper

1979 Knowles and Knowles 1989 Willemot, 1975) mechanical injury (Theologis and Laties, 1981), and senescence (Dhaulsa et al , 1981) Decreases in linoleic acid and increases in linolenic acid in phospholipids or glycolipids have been observed in wheat (Farkas et al 1975), rye (Clarkson et al , 1980 Farkes et al 1975), apple (Ketchie and Kuiper, 1979) and potato (Knowles and Knowles, 1989, Spychalla and Desborough 1990) when subjected to cold stress

The formation of peroxides and the breakdown of lipids are the major reactions in potatoes caused by irradiation (Hayashi et al 1992) Irradiated potatoes exhibited effects similar to those in cold-stressed potatoes, i e an increase in linolenic acid and decrease in linoleic acid and an increase in membrane permeability (Hayashi et al , 1992) However it is believed that the mechanisms of increased membrane permeability are different between cold-stressed and gamma-irradiated potatoes, since the increased rate of leakage (which occurred one hour after irradiation) was not correlated with the change in polar lipids (Hayashi et al. 1992)

Softening of fruit may be due, at least partially, to the loss of cellular turgor after irradiation induced damage to the cellular membranes (Hatton et al , 1984 Srb and Hluchovsky, 1963) Sutherland et al. (1967) reported that membranes may be altered by irradiation, leading to physiological changes. It has been reported by Skou (1983) that an enhanced rate of leakage of ions was observed in irradiated carrots An immediate increase in the rate of leakage of electrolytes from discs of irradiated potatoes was also observed following irradiation, which then continued to increase for 2 days, and

decreased gradually after that with the rate of leakage a function of irradiation dose (Hayashi et al , 1992)

Irradiation Effects on Proteins and Enzymes

It has been suggested that PME activity might be a controlling factor in the solubility of pectins, as it is believed that the deesterification of pectins by PME must precede the glycosidic hydrolysis by PG (Jansen and McDonnel 1945) The activities of PG and PME enzymes were not significantly affected immediately following irradiation of mature-green tomato fruit (Yasir et al., 1987) The activity of PG was observed to decline during storage in fruits irradiated at 1 0 kGy or higher (Yasir et al , 1987) Rouse and Dennison, (1968) reported that the activity of PME in irradiated orange and grapefruit was higher than the control fruit during storage and that softening in the storage was slow compared to the immediate rapid softening following irradiation

It was found that the activity of PME in irradiated pear and peach fruits increased when extracted immediately following lower dose of irradiation and decreased after 4 days in storage (Somogyi and Romani, 1964) Irradiation caused an increase in PME activity in orange juice obtained from fresh oranges irradiated at 1 0, 2 0 or 3 0 kGy (Dennison et al , 1967) When the fruit were stored for 7 days, a decrease in the PME activity was observed whereas a slight increase was observed in the non-irradiated fruits (Dennison et al , 1967) A decrease in PME activity was observed in the peel of orange fruit, while an increase was observed in the membrane as a function of increasing irradiation dose (Rouse and Dennison, 1968) In the juice sacs of both orange and grapefruit the PME activity was decreased by irradiation at 1 5 kGy, but increased at 3 0

kGy (Rouse and Dennison, 1968). Somogyi and Romani (1964) reported that the activity of PME in cherries increased immediately after irradiation with 2.0 or 5.0 kGy but decreased 4 days following irradiation.

The physical and chemical changes in bovine serum albumin (contained 5% water) induced by irradiation have been extensively studied by Alexander and Hamilton (1960). Their studies revealed that there was no alteration in the molecular weight of the protein to be associated with the increased amount of the protein due to the increase in the shape of the protein. The increase in the sedimentation constant can be explained as an opening of the protein molecule as its chemical reactivity increased. In the native molecule, the disulfide bonds are unavailable for any chemical reaction, whereas in a molecule denatured by heat or any other means, some of these disulfide bonds become susceptible to attack by oxidizing and reducing agents (Alexander and Hamilton, 1960). There was no evidence to indicate any alteration of the peptide bonds (Alexander and Hamilton, 1960).

Most of the data related to irradiation of proteins in aqueous solutions support the view that the free radicals produced in water are responsible for changes in the shape of the protein molecules (e.g. unfolding), facilitating the aggregation process and subsequent increases molecular weight. It has been postulated that irradiation has no effect on cell wall proteins, in agreement with results obtained by Quaranta and Piccini (1984). Irradiation of mesocarp tissue of cucumber pickles with doses between 0.5 and 1.0 kGy resulted in the reduction of the degree of esterification (DE) of pectic substances (Howard and Buescher, 1989). It is presumed that demethylation of pectic substances of irradiated

cucumber pickles is due to the increased activity of PME (Howard and Buescher 1989) Activation of PME by ionizing irradiation has been reported in cherries and citrus fruits (Somogyi and Romani, 1964, Rouse and Dennison, 1968)

CHAPTER 3
IRRADIATION-INDUCED SOFTENING OF TOMATO FRUIT

Introduction

Texture is an important quality parameter that often influences the manner in which horticultural commodities are handled after harvest. Collectively, texture has a direct influence on organoleptic perception and is of importance as a factor in the ability of the commodity to withstand the stresses of postharvest handling. It often dictates early harvest in order to avoid damage that might limit the postharvest life of the fruit.

In addition to the normal textural changes occurring as an inherent component of ripening, softening can also occur in response to certain postharvest operations, including irradiation. While irradiation is believed to prolong the shelf life of many fruits, one common consequence is the development of undesirable and/ or abnormal textural changes (Maxie et al. 1971, Kader, 1986, Urbain, 1986). The irradiation threshold at which deleterious changes are observed varies significantly for various plant tissues (Glegg et al. 1956).

Effects of irradiation on textural properties have been reported for numerous fruits including pear, peach, and nectarine (Maxie and Sommer. 1963), apple (Glegg et al 1956, Boyle et al., 1957, Phillips et al., 1960, Eric et al., 1970), lemon and orange (Maxie and Sommer, 1963), plum and raspberry (Truelsen, 1963), grape (Maxie et al., 1964),

strawberry (Cooper and Salunkhe, 1963, Johnson et al ,1965, Brecht et al , 1992), sweet cherry (Massey et al , 1965), and other organs, including carrot roots (Skou, 1963)

Irradiation-induced softening in tomato fruit has been reported to occur immediately following irradiation (Bramlage and Lipton, 1965, Abdel-Kader et al , 1968c Ahmed et al., 1972, and Yasir et al , 1987) However the range of techniques used in the above studies in preparing the fruit materials, and/ or in measuring firmness, make it difficult to compare the results from these different studies

In the normal ripening of tomato fruit, PG [endo(1-4) galacturonase] (EC 3 2.1 15) has been considered to be the primary enzyme involved in softening However, a considerable amount of evidence has been presented suggesting that PG is not solely responsible for cell wall structural changes occurring during normal ripening (Tucker and Grierson, 1982, Huber, 1984, Seymour et al., 1987, Smith et al , 1988) Such evidence suggests the possibility that other mechanisms and/or cell wall hydrolases such as glycosidases might be involved in cell wall degradation For example, β-galactosidase is present during early softening in a variety of fruits (Fischer and Bennet, 1991) and has been implicated in the degradation of cell wall galactans of tomato fruit (Pressy, 1983)

Little information is available on the effect of irradiation on PG activity No immediate effect of irradiation on PG enzyme activity following irradiation at 0 5 kGy and higher was observed (Yasir et al , 1987) However, after storage for 3 or 6 days a reduction in the activity was noted at 1 0 kGy and higher and a recovery at 0 5 kGy (Yasir et al., 1987). On the other hand PME an enzyme that carries out demethylation at the C6 position of methylegalacturonic acid residues, has been reported to be present in green

fruit and its activity to increase two to three fold during softening (Grierson et al, 1981, Hobson, 1963) Although PME activity is not correlated directly with the softening rate, it might influence cell wall degradation by other enzymes, especially PG (Tucker et al , 1982)

A number of researchers have suggested that irradiation-induced softening might result, in part, from a loss of cell turgor due to damage to cellular membranes (Srb and Hluchovsky, 1963, Hatton et al , 1984). Skou (1963) reported an enhanced rate of ion efflux in tissue discs from irradiated carrots An immediate dosage dependent, increase in the rate of electrolyte leakage was also observed in discs prepared from irradiated potato tubers (Hayashi et al., 1992)

The objectives of the current study were to examine the effects of ionizing irradiation on the textural properties of mature-green and ripening (pink) tomato fruit. Firmness, a component of texture, can be estimated using a number of compression and/or deformation techniques and these have been addressed in detail by Jackman and Stanley (1992a, 1992b) In the present study, estimates of texture changes were determined via deformation analysis of intact fruit, providing a composite determination of whole fruit effects, and by determining the bioyield or tissue failure parameters in excised pericarp discs (Ahrens and Huber 1990) Also measured were the effects of ionizing radiation on the net electrolyte efflux and on the cell wall enzymes PG, β-galactosidase, and PME.

Materials and Methods

Plant Material

Tomato (*Lycopersicon esculentum* L cv Sunny) fruit for the first two experiments were harvested at the mature-green and pink stages of development from commercial fields near Ruskin, FL , or from plots maintained at the UF Gulf Coast Research and Education Center at Bradenton, FL For these experiments, the fruit were transported to the Food Technology Service, Inc irradiation facility at Mulberry FL within 3-4 h after harvest In a third experiment, mature-green and pink fruit were obtained from a commercial packing house in Ft Pierce, FL and transported to Gainesville Fruit were selected for uniformity of size and freedom from defects, surface sterilized with 1 mM NaOCl for 1 minute, rinsed in tap H_2O followed by deionized (di) H_2O, and dried with paper towels or air-dried at 23°C (± 1)

Irradiation Treatment

For the first two experiments, irradiation was performed at Food Technology Service Inc , Mulberry, Fl using gamma-rays emitted from a Cobalt-60 source at doses of 0, 0 73 ± 0 11, or 2 21 ± 0 22 kGy for the first experiment, and 0, 0 825 ± 0 095, or 1.53 ± 0 12 kGy for the second experiment. Tomato fruit were placed in standard shipping cartons, which, in turn, were maintained on a rotating table during irradiation at the dosages mentioned above. Dosimetry, monitored spectrophotometrically by Food Technology Service staff, revealed a variation (range) of doses among the different locations within a given carton. Since more than one carton was used for each treatment, the fruit within a particular treatment from different cartons were combined and a range

of irradiation doses was given to each treatment that reflect the actual doses received The fruit were selected randomly from each treatment for post-irradiation measurements

In the first experiment, fruit were maintained at 20°C for 8 (pink) or 10 (green) days following irradiation. At selected intervals, fruit were removed for firmness determinations. In the second experiment, one of the objectives was to establish the immediate, short term and long term effects of irradiation on fruit firmness and electrolyte efflux In this experiment, fruit were placed in crushed ice immediately following irradiation to prevent indirect or secondary injury symptoms and also to retard compensatory repair responses following the irradiation treatment (Romani, 1984)

For the third experiment, irradiation was performed using an electron beam X-ray source at the Florida Department of Agriculture and Consumer Services Division of Plant Industry (DPI) facilities in Gainesville at 0, 0 72 ± 0 11, or 1 41 ± 0 15 kGy (dosimetry was conducted by DPI staff) Tomato fruit were placed in a 24-cell polyester tray one fruit per cell and four trays were placed in a standard 25-lb tomato carton. Since the DPI facilities did not allow automated rotation of the irradiated target after receiving one-half of the desired dosages, the cartons were turned 180° before completing the irradiation exposure Following irradiation, the fruit were treated as described above

Firmness Determinations

In measurements of whole fruit firmness, fruit were selected for uniformity of size. Firmness was determined using a Cornell firmness device (Hanson, 1952) as modified by Gull (1987) Individual fruit were placed on their side, a 1-kg weight was applied and deformation was measured after 5 sec Measurements were taken at three

points along the equatorial plane of the fruit, avoiding regions subtended by radial pericarp walls. In the first experiment, the initial firmness determinations were performed 5 hr following irradiation and again at daily intervals for 6 days for the pink and 12 days for the mature-green fruit. In the second experiment, initial measurements were performed within 5 hr of irradiation treatment and at daily intervals over 8 days for the pink and 10 days for the mature-green fruit.

In the third experiment, firmness of irradiated mature-green and pink fruit stored at 20°C was monitored in excised pericarp tissue (Ahrens and Huber 1990). Pericarp discs (1 cm diameter) were prepared from external tissue using a cork borer and trimmed to 3 mm in thickness by removing the skin and part of the internal pericarp with razor blades affixed 3 mm apart between plexiglass plates. A total of 12 discs from three fruit were used for each firmness measurement. Discs were tested using an Instron Universal Testing Instrument (Model# 1132, Instron Corporation, Canton, MA) equipped with a 50-kg load cell. Crosshead speed was 10 cm per min. Discs, placed in a plastic petri dish, were compressed to the point of tissue failure, or bioyield, with a 7.5 mm diameter probe (convex tip). The peak height over the course of travel was recorded.

Preparation of Alcohol-Insoluble Solids

Tomato fruit were cut radially, the gel and placental tissue removed, and 100 g of pericarp tissue were then homogenized in 400 ml of 100% cold ethanol (final ethanol concentration of 80%) using a Polytron (Brinkmann Instruments, Westbury, N.Y.) set at speed # 7 for 3 minutes. The alcohol insoluble solids (AIS) were prepared as described by (Huber 1991) with slight modifications (Huber and O'Donoghue, 1993). One-hundred

ml of the EtOH suspensions (homogenized pericarp tissue in 80% EtOH) were filtered through Miracloth (Calbiochem Corp, La Jolla, CA) in a Buchner funnel under aspiration and washed with 100 ml of 80% EtOH. The residue was recovered from the filter paper and transferred to 45 ml Tris-buffered phenol, pH 7.0 (Huber 1992) for 30 min at 23°C (±1) while stirring at low speed. The suspension was readjusted to 80% EtOH and held at 20°C for 30 min to facilitate the re-precipitation of the AIS. After another filtration through Miracloth and washing with 200 ml 80% EtOH, the residue was transferred to 100 ml chloroform:methanol (1:1 v/v) for 30 min at 23°C (±1) while stirring at low speed. The suspension was then filtered, washed with 200 ml acetone, partially dried under aspiration, and transferred into a glass petri dish and placed in an oven at 34°C for 12 hr. The AIS preparations were stored in a desiccator at 23°C (±1) until needed.

Extraction and Assay of Polygalacturonase, Pectinmethylesterase, and β-galactosidase

Salt-extractable proteins (including enzymes) were prepared from alcohol-insoluble substances (AIS) as described by Huber and O'Donoghue (1993). Aliquots of pericarp tissue (3 g fresh weight) in 15 ml 80% ethanol (EtOH) were centrifuged at 15,000 x g for 20 min at 4°C using a Beckman Model J2-21 centrifuge and Beckman J-20 rotor (Beckman Instruments Inc, Irvine, CA). The supernatant was discarded and the pellet was resuspended in 25 ml cold 80% EtOH and centrifuged again under the same conditions. The final pellet was suspended in 15 ml 50 mM Tris, 1.2 M sodium chloride (NaCl), pH 7.0 and incubated for 30 min in an ice bath. The suspension was centrifuged at 15,000 x g for another 20 min at 4°C. The supernatant was then filtered through

Miracloth, the pellet discarded, and the filtered protein extract placed in ice until assayed.

Polygalacturonase (PG) activity was determined using polygalacturonic acid (Sigma) in 30 mM sodium acetate (NaOAc), pH 4 5 A 0 1 ml (2-3 µgs protein) aliquot of active or inactive (i.e boiled in H_2O for 10 min) enzyme (protein extract) were added to 0 5 ml substrate (1 mg) in a test tube and incubated at 34°C for 30 min The reaction was terminated by heating in boiling H_2O for 10 min. Activity was determined reductometrically using the Milner-Avigad assay for uronic acid reducing groups (Milner and Avigad 1967) Absorbance was measured at 600 nm using a Beckman DU-20 spectrophotometer equipped with Quant II Soft-Pac$_{TM}$ Module (Beckman Instruments Inc, Irvine, CA) Enzyme activity was expressed as umol galacturonic acid reducing equiv g tissue fresh weight $^{-1}$ min^{-1}

Measurement of PME activity was conducted by determining the amount of methanol released from polygalacturonic acid as described by Wood and Siddiqui (1971) with slight modifications. A 0 2 ml (4-6 µgs protein) aliquot of active or inactive enzyme (protein extract) along with 0 3 ml of substrate (0 2% of 90% esterified polygalacturonic acid from citrus) in 100 mM sodium acetate (NaOAc), pH 6 0 were incubated at 30°C for 60 min The reaction was terminated by adding 0 5 ml of 2 N sulfuric acid (H_2SO_4) and 0.2 ml of 2% (w/v) aqueous potassium permanganate ($KMnO_4$) and swirling gently After incubation in an ice bath for 15 min 0 2 ml of 0 5 M sodium arsenite in 0 12 N sulfuric acid was added, followed by adding 0 6 ml of di H_2O After 1 hr at 25°C, 2 0 ml of 0 02 M acetylacetone (dissolved in a solution containing 2 M ammonium acetate and 0 05 M acetic acid) were added. After shaking the tubes were heated at 60°C for 15 min

and then cooled to room temperature. Absorbance was measured at 412 nm using a spectrophotometer (Shimadzu UV-VIS recording Spectrophotometer UV-160, P/N 204-04550 Shimadzu Corporation Tokyo, Japan)

Activity of the β-galactosidase was determined as described by Huber and Nevins (1981). A 0.1 ml (2-3 µgs protein) aliquot of the protein extract was added to 0.1 ml substrate (25 mM p-nitrophenyl β-d galactopyranoside in 10 mM of sodium acetate buffer at pH 5.5) in a test tube containing 0.8 ml sodium acetate buffer. After incubation at 30°C for 30 min, the reaction was terminated by adding 2 ml of 200 mM sodium carbonate. Absorbance was measured at 400 nm

Electrolyte Efflux

Electrolyte efflux was determined according to the modified protocol of Whitlow et al. (1992) using a YSI Modal 31A conductivity bridge equipped with a YSI Model 3403A conductivity electrode (Yellow Springs Instruments Inc. Yellow Springs, OH). Pericarp discs (1 cm diameter) were prepared using a cork borer from 18 tomato fruit (5 discs per fruit, 3 fruit per treatment), rinsed briefly in di H_2O, then transferred to 14 ml of 300 mM mannitol and placed on a shaker. Conductivity was determined after 1 and 3 hr (for fruit irradiated at 0, 0.73 ± 0.11, or 2.21 ± 0.22 kGy), at 2 and 4 hr (for fruit irradiated at 0, 0.72 ± 0.11, or 1.41 ± 0.15 kGy) intervals and total tissue electrolytes determined after subjecting the discs to a freeze-thew cycle. Net efflux was expressed as a percentage of total tissue electrolyte content

Statistical Analysis

Data from the three irradiation treatments were analyzed as a completely randomized design with three replicates Data obtained from each fruit maturity stage were analyzed separately using Analysis of Variance (ANOVA), and means were separated by the Least Significant Difference method at the 0 05 level

Results and Discussion

Firmness of Whole Fruit as Affected by Irradiation

A significant decrease in firmness was apparent within 5 hr following irradiation in the first experiment for mature-green tomato fruit irradiated at 0, 0 73 ± 0 11, or 2.21 ± 0 22 kGy (Fig 3-1), or 24 hr in the second experiment for fruit irradiated at 0, 0 825 ± 0 095, or 1 53 ± 0 12 kGy (Fig 3-2), indicating that irradiation affected texture components rather directly This initial loss of firmness noted early following irradiation was not as marked as was reported for green tomato fruit irradiated at 0 to 6 0 kGy (Abdel-Kader et al., 1968c), or green fruit irradiated at 0 to 5 0 kGy (Bramlage and Lipton, 1965) Additionally the texture changes noted were not as dramatic as those reported by Yasir et al , (1987), who reported that mature- green fruit treated at 2.5 or 5 0 kGy softened to an extent comparable in magnitude to that occurring during ripening of non-irradiated fruit However the more dramatic effects of irradiation on firmness mentioned above (Abdel-Kader et al., 1968c, Bramlage and Lipton, 1965, Yasir et al 1987) compared to the results in this study might be explained by the very high irradiation doses used in those experiments. In the present study, no differences in

firmness were detected 2 days after irradiation and for up to 6 days during storage, either between the control and the irradiated fruit or among fruit irradiated at different doses in the first experiment (Fig. 3-1). However, significant differences were observed between the control and the higher irradiation dose treatment on the second day, and between the control and lower irradiation dose treatments compared to the higher dose in the second experiment on the third day (Fig. 3-2). Significant differences in firmness between irradiated and non irradiated fruit were generally maintained during the late post-irradiation period, particularly the last 3 (Fig. 3-1) or 4 days (Fig. 3-2). This pattern of texture loss is in contrast to the progressive loss of firmness reported by Ahmed et al. (1972) and Bramlage and Lipton (1965). Ahmed et al. (1972) reported that firmness loss was more pronounced during the first 5 days than at 7 days after irradiation, whereas in this study a significant decline in firmness was observed after the sixth day that was maintained for the remaining period of storage. No recovery in textural properties was noted as was reported for pear fruit irradiated at 3.0, 6.0 or 9.0 kGy (McArdle and Nehemias, 1956), or tomato fruit irradiated at 0.5, 2.0, 3.0, 4.0 or 6.0 kGy (Abdel-Kader et al., 1968c) and 0.5, 1.0, 2.0 or 5.0 kGy (Yasir et al., 1987).

The data in this study indicate that irradiation-induced firmness loss in mature-green tomato fruit is, in part, irreversible, with firmness differences remaining significant during the latter period of post-irradiation storage. Furthermore, the firmness decrease of irradiated mature-green fruit was dosage independent within the range used here.

The firmness of irradiated pink fruit measured immediately following irradiation was not significantly different from that of the control fruit (Fig. 3-3). Pink fruit

irradiated at 0 73 ± 0 11or 2 21 ± 0 22 kGy were significantly less firmer than control fruit on the second day (Fig 3-3) Significant differences in firmness were also noted on the third and fifth days for the pink fruit irradiated at 0 825 ± 0 095 or 1 53 ± 0 12 compared to firmness of the control fruit (Fig 3-4) There were no significant statistical differences in firmness loss detected between irradiated pink fruit and control in all other firmness measurements except those mentioned above (Fig 3-3 and 3-4) The effect of irradiation on pink fruit was of significantly lower magnitude than was observed for mature-green fruit Abdel Kader et al (1968c) also reported a decrease in the magnitude of the effect of irradiation on firmness when the fruit subjected to irradiation where at an advanced stage of ripening, while Ahmed et al. (1972) and Bramlage and Lipton (1965) observed the opposite effect.

Firmness of Pericarp Discs as Affected by Irradiation

As was noted for firmness of intact fruit, irradiation-induced firmness loss was apparent in pericarp discs excised from mature-green tomato fruit within 24 hr following irradiation at dosages of 0 72 ± 0 11 or 1 41 ± 0 15 kGy (Fig 3-5) More so than with whole fruit firmness treods the effect of irradiation determined using discs became more pronounced during the post-irradiation fruit storage period and was more clearly dosage dependent, with the higher dosage (1 41 ± 0 15 kGy) resulting in significantly enhanced firmness loss relative to the lower dosage (Fig 3-5) While the bioyield force in discs from pericarp tissue of the control fruit decreased progressively throughout the storage period those from the irradiated fruit pericarp discs decreased sharply reaching a minimum by the fifth day compared to the control (non-irradiated) treatment. A slight

increase was noted in the bioyield forces on the sixth and seventh day for both irradiation levels; however, these values were still lower than those of the control, and can not be interpreted as an elastic recovery response in fruit firmness. After 6-7 days, firmness of discs from the high-dosage fruit exhibited little additional change. By the seventh day of storage, values for firmness of discs from the low dosage fruit were comparable to those of the non-irradiated fruit, which, due to ripening-related softening, had converged with those of the low dosage fruit, whereas significant differences were still apparent between control and high dosage treatments through the tenth (last) day of this experiment (Fig. 3-5). The greater response of the outer pericarp to irradiation treatment compared to the whole fruit could be related to the contribution of the supportive internal, underlying tissues to firmness when using the intact fruit, whereas discs are composed primarily of a single tissue type. These results indicate that pericarp tissue is proportionally more dramatically affected compared to intact fruit as a unit and that the effect of irradiation on pericarp tissue texture is dosage dependent.

As observed with the green fruit, the effect of irradiation on the textural properties of pink fruit was much more pronounced in pericarp discs (Fig. 3-6) compared to whole fruit. The effect of irradiation as measured in pericarp discs from pink fruit showed no dosage dependency until the sixth and seventh days following irradiation, when significant differences were noted among treatments and loss of firmness was greater for the higher irradiation dose. Toward the end of the post-irradiation storage period for pink fruit, at which time whole fruit firmness was quite similar for the irradiated and control fruit (Figs. 3-3 and 3-4), irradiation effects were still highly significant in pericarp tissue

for both irradiation doses It is noteworthy that the marked effects of irradiation on pericarp tissue, clearly evident throughout the post-irradiation period (Fig 3-6), were only minimally apparent when firmness was expressed as a composite, whole-fruit parameter

Alteration of Cell Wall-Bound Enzymes by Irradiation

Polygalacturonase (PG) The greater effects of irradiation on pericarp tissue firmness compared to whole fruit firmness suggested a possible role for polygalacturonase in the irradiation effects This enzyme is differentially distributed in tomato fruit, being present in all pericarp regions and largely absent from the internal locule tissue (Huber and Lee, 1986) Figures 3-7 and 3-8 illustrate the PG activity in mature-green and pink fruit, respectively No enzyme activity was detected at the time of irradiation of mature-green fruit, consistent with reports that PG is absent at this developmental stage (Hobson, 1964, Buescher and Tigchelaar, 1975, Grierson et al 1987) By the third day of post-irradiation storage, PG was detected in non-irradiated fruit and in fruit treated at the low irradiation dose (0 72 ± 0 11 kGy) (Fig 3-7) Activity in mature-green fruit irradiated at the high dose (1 41 ± 0 15 kGy) was not detected until after 5 days of storage, after which time little additional accumulation of PG activity was observed in fruit from either irradiation treatment As Figure 3-7 illustrates, PG activity in control fruit increased dramatically during storage, consistent with the reports of progressive PG accumulation during normal ripening (Hobson, 1964) At the termination of the experiment with green fruit, PG activity in irradiated fruit was about 9% and 3% of control levels in the low and high dosages, respectively These data are in contrast to the

results reported by Yasir et al (1987) that there was no immediate effect on PG activity following irradiation, and that a recovery in the activity of the enzyme was noted in fruit irradiated at 0 5 kGy, while a reduction was observed in fruits irradiated at 1 0 kGy and higher (Yasir et al., 1987)

Lower levels of PG activity were detected 24 hr following irradiation in pink fruit at both dosages compared to the activity levels of the enzyme detected in the control fruit (Fig 3-8) Although PG activity increased over the course of storage in both control and irradiated pink fruit, the levels in the irradiated fruit remained lower than in the control The reduced activity of PG enzyme in irradiated pink fruit was not dosage dependent except for the first 24 hr following irradiation No significant differences were noted between the activity levels of the enzyme in the low versus high irradiation treatments after the first day following irradiation.

It is clear from these results that the effect of irradiation on the activity of PG is more pronounced in irradiated mature-green than irradiated pink fruit. Such an observation was also noted in regard to the firmness loss discussed earlier It is likely that the younger the tissue at the time of irradiation, the more severe the response will be

β-galactosidase. Significantly higher activity (18%) of β-galactosidase was observed 24 hr following irradiation in mature-green fruit receiving the higher dose compared to the activity levels of the control, while the low irradiation dose was intermediate (Fig 3-9) By the third day β- galactosidase activity had increased sharply in all treatments with no significant differences among them β-galactosidase activity of

the control fruit increased slightly (13%) thereafter, while there were sharp declines of 69% and 74% in the activity of the low and high irradiation treatments, respectively, by the fifth day The activity levels in the irradiated fruit were 37% and 32% lower than the levels of the control on the fifth day Although another increase was noted in the activity of β-galactosidase in irradiated fruit on the seventh and ninth days, the activity in the irradiated fruit was still lower than the control, even though the latter decreased on the seventh day By the ninth day, the activity of the enzyme was at its maximum for all treatments and there were no significant differences

In pink fruit, the effect of irradiation on the β- galactosidase activity was dramatic for the first 3 days following irradiation as higher levels of activity (35% and 86%) were noted in the low and high dose irradiation treatments, respectively, 24 hr following irradiation compared to the levels in the control fruit (Fig 3-10) This was followed by a further sharp increase in enzyme activity in irradiated and non irradiated (control) fruit on the third day The increased activity levels noted were 40 and 72% higher in the low and high dose treatments, respectively, compared to the control treatment On the fifth and seventh days a decrease and leveling off in the activity of the enzyme in irradiated fruit was noted, while a gradual slight increase in the activity of the enzyme through the fifth day was noted in the control fruit. The effect of irradiation on β-galactosidase activity in irradiated pink fruit was clearly dosage dependent, evident by the significant differences noted between the irradiation doses for the first 3 days

These data indicate that the effect of irradiation on β-galactosidase activity was not as pronounced as the effect noted for PG enzyme activity in irradiated mature-green fruit. β-galactosidase activity was present in detectable levels in irradiated fruit and was somewhat comparable to the levels of the control fruit. In pink fruit, irradiation treatment was noted to enhance β-galactosidase activity in irradiated fruit rather than suppressing it as noted in irradiated mature-green fruit. These data support the suggestion that the impact of irradiation on mature-green fruit was more severe than the effect of irradiation on pink fruit. The involvement of β-galactosidase in the excessive softening noted in this study in the pericarp tissues of irradiated fruit is not clear. It has been suggested that loss of firmness during normal ripening and storage of apples and avocado was associated with increased levels of β-galactosidase activity (Berard et al., 1982, De Veau et al., 1993). Increased levels of β-galactosidase activity were also noted in ripening tomato fruit (Pressey, 1983). It was shown that an isolated isoenzyme (β-galactosidase II) was capable of hydrolyzing the galactose-rich polysaccharides isolated from tomato fruit (Pressey, 1983). These findings led to the conclusion that β-galactosidase is responsible for the loss of galactose noted during normal ripening of tomato fruit (Pressey 1983). In the present study, β-galactosidase activity in irradiated pink fruit exhibited a dramatic rise following irradiation, while that of irradiated mature-green fruit exhibited a reduction instead. Nevertheless, loss of firmness was more drastic in irradiated mature-green fruit than in pink fruit. This situation would preliminarily imply that β-galactosidase had no role in the irradiation-induced softening noted in this study.

Pectinmethylesterase (PME) Higher extractable PME activity (20%) in the high dose irradiation treatments of mature-green fruit compared to that of the control fruit was noted 24 hr following irradiation , with the low irradiation dose treatment intermediate (Fig 3-11) A decrease of 12 and 32% in PME levels of the control fruit occurred on the third and fifth days, respectively, relative to the levels detected after 24 hr This was associated with a slight reduction in the levels of PME activity in the low irradiation dose treatment on the third and fifth days, which were still higher than the control for the same period of time, whereas dramatic increases of 39% and 99% in PME levels occurred in the high irradiation dose treatment on the third and fifth days, respectively, relative to the values obtained for the control on the same days (Fig 3-11) On the seventh day there was a dramatic increase of 76% in PME activity in the control, while there was only a slight increase in the activity of PME in the low irradiation dose treatment and a slight reduction in the high irradiation dose (Fig 3-11) On the seventh day there were no detectable differences in PME activity among the treatments, while on the ninth day, PME activity was undetectable in the control and low irradiation dose, and the high irradiation dose had trace PME activity

In a manner similar to that noted in mature-green fruit, the effect of irradiation on the PME activity in pink fruit was dramatic 24 hr following irradiation (Fig 3-12), with 18% and 47% higher levels of PME activity in the low and high dosage treated fruit, respectively, relative to the control fruit. Thereafter the pattern of PME activity was in contrast to that noted in mature-green fruit While levels of PME activity continued to increase in the control fruit, or remain relatively constant at the lower irradiation dose, a

dramatic decline (47%) in PME activity was observed at the higher irradiation dose relative to the control on the third day. Further declines in the activity of the enzyme in the high irradiation dose treatment persisted throughout the storage period, whereas the activity in the control and the low irradiation dose treatments increased slightly on the fifth day. A noticeable decrease in PME activity (48% and 28%) was observed in the control and low irradiation dose treatments on the last day, at which time there were no significant differences between the two treatments. Significant differences between the higher and lower irradiation doses persisted throughout the experiment.

These results clearly indicate the dramatic effect of irradiation on the level of PME activity in irradiated fruit, and that the effect was also dosage dependent. Fruit treated with the high irradiation dose in both maturity classes were severely affected, but in totally opposite trends. While the effect of irradiation on PME activity in mature-green fruit was reflected by a steady increase during the post-irradiation storage period, irradiated pink fruit were characterized by a steady decrease in PME activity. The effect of the lower irradiation dose was not as dramatic as that of the high irradiation dose in both maturity classes. However, at the low irradiation dose, mature-green fruit were more affected than pink fruit.

The higher activities of PME following irradiation of mature-green and pink fruit noted after 24 hr were in agreement with the early findings of Somogyi and Romani (1964) for sweet cherries irradiated at 2.0 or 5.0 kGy, and by Dennison et al. (1967) for oranges irradiated at 1.0, 2.0, or 3.0 kGy. However, both of these reports noted a decrease in extractable PME activity after storage of sweet cherries for more than 4 days

(Somogyi and Romani, 1964) and oranges for 7 or more days (Dennison et al., 1967). It seems that the trend of the irradiation effect on PME activity noted in this study in pink fruit irradiated at the higher irradiation dose is in agreement with these early findings, when higher PME levels were detected within 24 hr following irradiation followed by a sharp decline thereafter. However, the trends of the irradiation effect on mature-green fruit irradiated at both doses, and the lower irradiation dose treatments in pink fruit, seem to contradict the findings mentioned above. As noted in the results above, PME activity in the low irradiation doses in both maturity classes either increased slightly or remained constant for the entire post-irradiation storage period. Furthermore, PME activity in the high irradiation dose was noted to increase following irradiation relative to the control and low irradiation dose treatments for the first 5 days. Kertesz et al. (1956) showed that methylester values did not change significantly in response to irradiation and concluded that deesterification was not a major event in the irradiation-induced degradation of isolated pectins.

Electrolyte Efflux

Net electrolyte efflux of discs prepared from the outer pericarp of tomato fruit irradiated with gamma rays at 0 (control), 0.73 ± 0.11 (low dose), or 2.21 ± 0.22 kGy (high dose), and X- rays at 0 (control), 0.72 ± 0.11 (low dose), or 1.41 ± 0.15 kGy (high dose) is shown in Tables 3-1 and 3-2. No differences in electrolyte efflux were noted for discs from control and irradiated mature-green tomato fruit in short (1 hr) incubations in isotonic mannitol (Table 3-1), whereas a significant difference was observed between the control and the higher irradiation dose after incubation of mature-green discs for 2 hr

(Table 3-2) Following longer incubation (3 or 4 hr) in mannitol, increases of 21% (Table 3-1) and 11% (Table 3-2) in net efflux were noted for discs from mature-green fruit irradiated at the high doses, relative to the control treatments.

Table 3-1 Electrolyte efflux[1] in pericarp discs of mature-green and pink tomato fruit following irradiation at 0 (control),0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose).

	Treatment	1 hr	3 hr
Mature green			
	Control	15 4a[2,3]	24 3a
	Low dose	13 9a	23 5a
	High dose	15 2a	29 4b
Pink			
	Control	14 9a	23 4a
	Low dose	31 8b	37 9c
	High dose	37 9b	52 0d

[1] Electrolyte efflux values are expressed as percentage of total electrolyte content
[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0 05 level by LSD method
[3] Means were determined from three separate sets of pericarp tissue from different fruit within a particular treatment (each set consisted of 15 discs from 3 fruits)

In contrast to the discs from mature-green fruit, discs from irradiated pink fruit showed dramatic increases in the electrolyte efflux of 113 and 154% in discs from the low and high doses, respectively relative to the control treatment after incubation for 1 hr (Table 3-1) Sharp increases of 62 and 122% in the electrolyte efflux were observed in

pink discs from the low and high doses, respectively, relative to the control treatment after incubation for 3 hr (Table 3-2) In the next experiment, discs from the low and high

Table 3-2 Electrolyte efflux[1] in pericarp discs of mature green and pink tomato fruit following irradiation at 0 (control) 0 73 ± 0 11 (low dose) or 2 21 ± 0 15 kGy (high dose)

	Treatment	2 hr	4 hr
Mature green			
	Control	19 3a[2,3]	30 4a,b
	Low dose	20 9a	28 4a
	High dose	28 4b	33 7b
Pink			
	Control	22 4a	37 4a
	Low dose	36 3b	43 0a,b
	High dose	43 4c	48 8b

[1] Electrolyte efflux values are expressed as percentage of total electrolyte content.
[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0 05 level by LSD method
[3] Means were determined from three separate sets of pericarp tissue from different fruits within a particular treatment (each set consisted of 15 discs from 3 fruits)

doses exhibited increases of 62 and 94% respectively, after 3 hr and 16 and 30% respectively after incubation for 4 hr relative to the corresponding control treatment

These results clearly demonstrate the effect of irradiation on the permeability of membranes as evident by the enhanced electrolyte efflux from irradiated tomato fruit, indicating the occurrence of membrane damage Furthermore, irradiated pink fruits

apparently were more severely affected than irradiated mature-green fruits in both experiments. The increase in electrolyte efflux was also dose dependent as evident by the higher percentage of electrolyte efflux in discs irradiated with higher doses. A remarkable observation was the higher relative electrolyte efflux values noticed in the irradiated disks compared to control discs at first incubation time than those noted for the second incubation, which indicates that the membrane damage incurred as a result of irradiation was both rapid and severe.

Temporal Relationship between Enzyme Activity Trends and Firmness of Pericarp Discs

Mature-green fruit. Absence of PG activity, and relatively high levels of PME and β-galactosidase activity were associated with a remarkable loss in pericarp firmness in the irradiated fruit during the first 24 hr following irradiation (Fig. 3-13, A, B, and C), as well as with less pronounced electrolyte efflux in pericarp tissue of irradiated mature-green fruit (Table 3-1 and 3-2). Firmness continued to decrease sharply in the pericarp tissue of irradiated mature-green fruit on the third day, with either the absence of detectable PG (Fig. 3-13, C), or lower activities of PG and PME than the control fruit (Fig. 3-13, B), but with a noticeable increase in the activity of β-galactosidase. A sharp decline in the β-galactosidase activity in both irradiation treatments was associated with either increased (Fig. 3-13, C) or decreased (Fig. 3-13, B) PME activity on the fifth day, which was in turn associated with the appearance of PG activity in all treatments (but the activity in the irradiated fruit was lower than in the control). The appearance of PG in the

irradiated fruit coincided with a sharp decrease in firmness which was more pronounced at the higher irradiation dose (Fig 3-13, B and C) Firmness loss in all treatments continued to the seventh and ninth days, but was more severe in irradiated fruit pericarp than in the control pericarp The later firmness losses were accompanied by sharp increases in PG, β-galactosidase, and PME activities in the control, slight increases at the high irradiation dose, and a decrease in the low irradiation dose treatment

The pattern of the texture change in the pericarp of the control fruit was one of progressively increasing softening, which was associated with either steady levels or increases in β-galactosidase and PME activities and the appearance and increase of PG activity However, in irradiated fruits at both doses, the firmness loss was also progressive, but with a more severe trend than the control. This excessive loss of firmness was not accompanied by increases in PG activity (the putative key enzyme involved in softening) for the first 3 days in the high irradiation dose treatment, and was followed by slight increases that were not comparable to that of the control The activities of β-galactosidase and PME were higher than the control for the first 5 days and dropped considerably below the levels of the control for the last 2 days In addition, there were only slight differences in the electrolyte efflux values between the control and high irradiation treatments indicating that there were only slight effects of irradiation on the membranes These results imply that the loss of firmness was a result of the direct impact of irradiation on the tissue and was not enzymically mediated Furthermore, since no serious impact was imposed on the membranes the loss of firmness in irradiated mature-green fruit was presumably cell wall related

Pink fruit. In pink fruit, a reduction in PG activity in irradiated fruit was associated with an increase in PME activity and an increase in β-galactosidase activity in the lower dose treatment (Fig 3-14-A B,C) A noticeable decrease in firmness occurred only in the higher irradiation dose treatment 24 hr following irradiation. These observations coincided with higher values of electrolyte efflux, especially in the higher dose treatment (Tables 3-1 and 3-2) Although PG activity increased in all treatments on the fifth day, no significant effect on firmness was observed in either irradiation dose treatment. This might indicate a possible correlation between β-galactosidase, which decreased sharply on day 5, and softening which also decreased (high dose) or at least did not increase (low dose) However, a slight recovery in β-galactosidase activity, with still increasing PG and PME activities in all of the treatments was noted to coincide with a sharp decrease in firmness in the higher irradiation dose treatment and a slight decrease in the lower dose treatment However while significant enzyme activity was present in all of the treatments most of the time, a considerable loss of firmness was clear in irradiated fruits compared to the control. Thus would further imply that loss of firmness was not enzymically mediated and was rather a direct consequence of irradiation The significant differences in the electrolyte efflux between the control and irradiated fruits indicates that membrane damage occurred It is presumed though that the loss of firmness in the pericarp of irradiated pink fruit was likely to result from irradiation directly affecting the structural features of the cell wall and membranes and indirectly affecting softening through altering enzyme activities

Color Development as Affected by Irradiation

In addition to the effect of irradiation on fruit firmness, a retardation of ripening-related pigmentation changes was noted for mature-green fruit during the initial 7 days following treatment (Fig 3-15 A, B, and C) The typical pigmentation changes in tomato fruit, involving loss of chlorophyll and accumulation of lycopene were disrupted or delayed in irradiated fruit compared to the control fruit (Fig 3-15 A, B and C) resulting in irregularly ripened fruit based on visual observation. This is in agreement with Bramlage and Liptoo (1965) who found that irradiation prevented the development of the red color of mature-green tomato fruit irradiated at 1 25 or 2 50 Abdel-Kader et al (1968 b) found that irradiation delayed color development for 4-8 days in irradiated mature-green tomato fruit stored at 20°C

Conclusions

An immediate loss of tissue firmness was observed in response to irradiation in mature-green and pink tomato fruit Irradiation-induced softening of mature-green tomato fruit was more severe compared to that induced in pink tomato fruit. This loss of firmness was associated with a significant effect on the three enzymes tested (a reduction in PG enzyme activity in both maturity classes and initial increases followed by decreases in PME and β-galactosidase) Therefore, we conclude that the mechanism of irradiation-induced softening might involve other enzymes than PG and/ or occurred as a direct effect of irradiation on the structure of the cell wall In addition, a significant increase in the electrolyte efflux was observed in irradiated pink tomato fruit whereas,

slightly significant differences were noted in irradiated mature-green fruit at the high irradiation dose only Thus we suggest that in addition to the cell wall-related mechanism that is believed to be involved in the loss of firmness in both mature-green and pink tomato fruit, another membrane-related mechanism is more likely to act on pink fruit

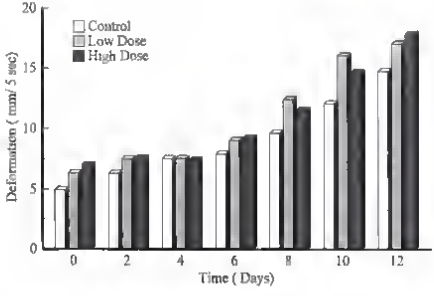


Figure 3-1 Firmness of intact mature-green tomato fruit following irradiation at 0 (control) 0 73 ± 0 11 (low dose) or 2 21 ± 0 22 kGy (high dose) Measurements were taken every two days over a 12-day period of storage at 20° C Higher values reflect softer fruit. LSD = 1 0

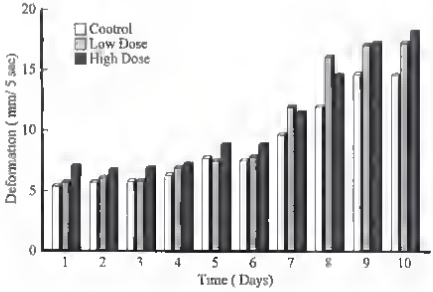


Figure 3-2 Firmness of intact mature green tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose) Measurements were taken every two days over a 12-day period of storage at 20° C Higher values reflect softer fruit. LSD = 1 0

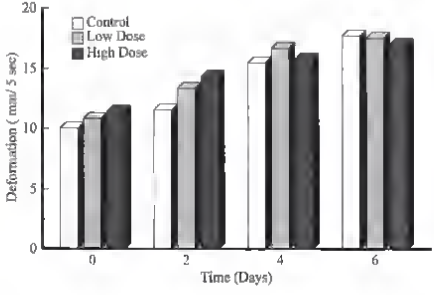


Figure 3-3 Firmness of intact pink tomato fruit following irradiation at 0 (control) 0 73 ± 0 11 (low dose), or 2 21 ± 0 22 kGy (high dose) Measurements were taken every two days over a 6-day storage period at 20°C Higher values reflect softer fruit LSD = 1 0

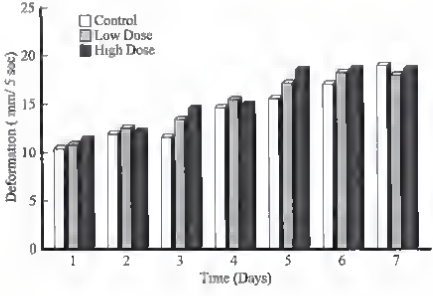


Figure 3-4 Firmness of intact pink tomato fruit following irradiation at 0 (control), 0.72 ± 0.11 (low dose), or 1.41 ± 0.15 kGy (high dose). Measurements were taken every two days x 7-day storage period at 20°C. Higher values reflect softer fruit. LSD = 1.0

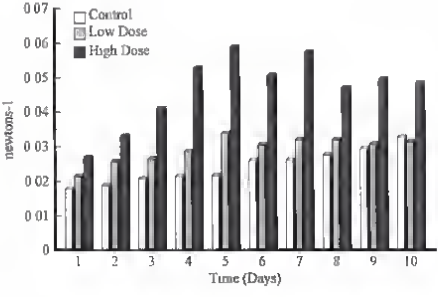


Figure 3-5 Firmness of discs from pericarp tissue of mature-green tomato fruit following irradiation at 0 (control) 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose) Measurements were taken every two days over a 10-day period of storage at 20°C Higher values reflect softer fruit. LSD = 0 0024

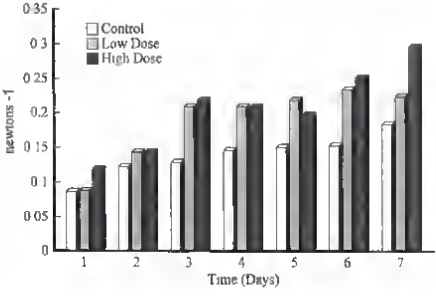


Figure 3-6 Firmness of discs from pericarp tissue of pink tomato fruit following irradiation at 0 (control) 0.72 ± 0.11 (low dose) or 1.41 ± 0.15 kGy (high dose). Measurements were taken every two days a 7-day storage period at 20° C. Higher values reflect softer fruit. LSD = 0.008

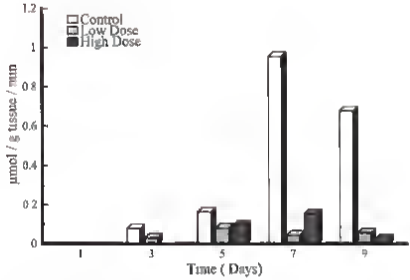


Figure 3-7 Total PG activity of mature-green tomato fruit following irradiation at 0 (control) 0 71 ± 0 11 (low dose) or 1 41 ± 0 15 kGy (high dose), and stored at 20° C for nine days Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit. Units are defined as µmol per g f w per min. LSD =0 02

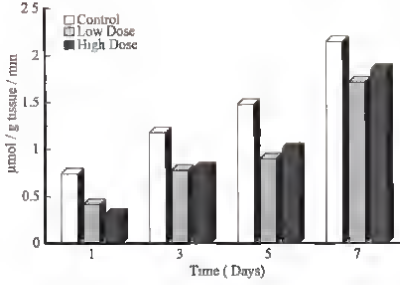


Figure 3-8 Total PG activity of pink tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose), and stored at 20 C for seven days Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit Units are defined as µmol per g f w per min LSD =0 2

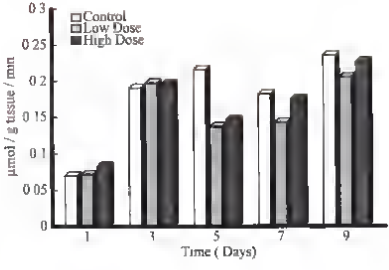


Figure 3-9 Total PME activity of mature-green tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose), and stored at 20°C for nine days Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit. Units are defined as µmol per g f w per min LSD =0 03

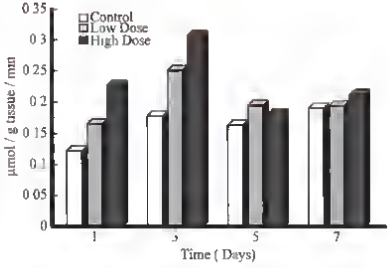


Figure 3-10 Total Beta-galactosidase activity of pink tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose) and stored at 20° C for nine days. Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit. Units are defined as µmol per g f w per min LSD =0 03

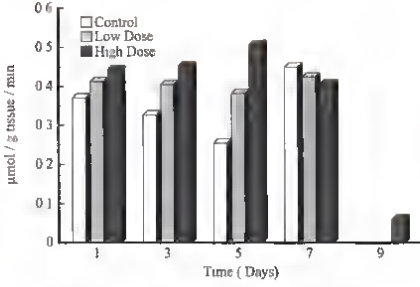


Figure 3-11 Total PME activity of mature green-tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose), and stored at 20°C for nine days Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit. Units are defined as µmol per g f w per min LSD =0 02

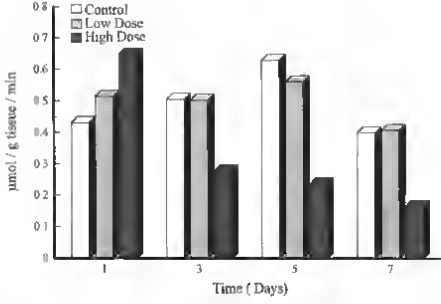


Figure 3-12 Total PME activity in pink tomato fruit following irradiation at 0 (control), 0.72 ± 0.11 (low dose), or 1.41 ± 0.15 kGy (high dose), and stored at 20° C for seven days. Samples were taken every two days from AIS preparation derived from pericarp tissue of the fruit. Units are expressed as μmol per gram fresh weight per min. LSD = 8.0

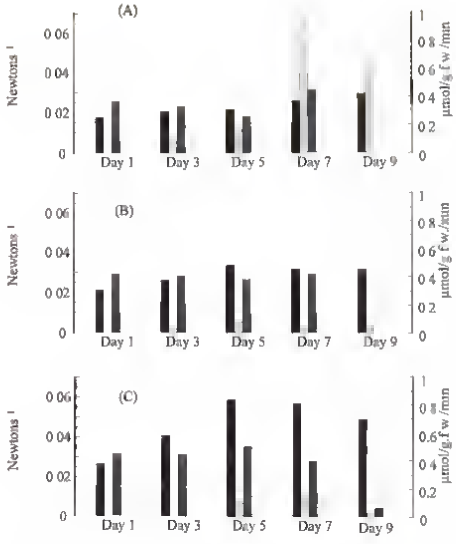


Figure 3-13 Temporal relationship between detectable enzyme activities of PG □ , PME ■ Beta-galctosidase , and firmness ■ of pericarp discs of mature green-tomato fruit following irradiationat 0 (A), 0 72 ± 0 11(B) or 1 41 ± 0 15 kGy (C) and storage at 20°C for nine days. Enzyme activity is expressed as µmol per gram fresh weight per min Higher Newtons values indicate softer tissue.

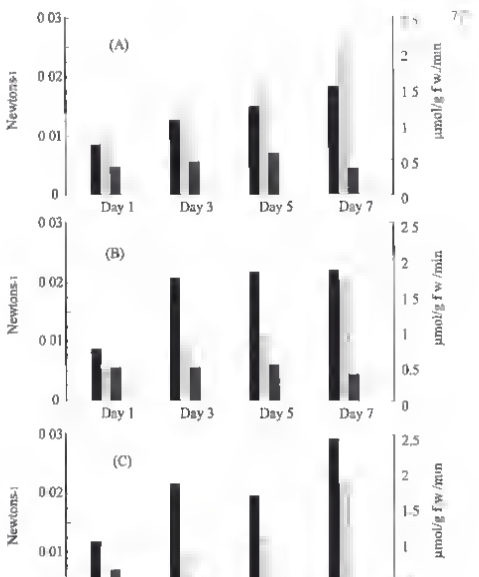
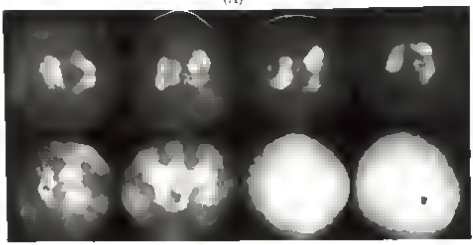


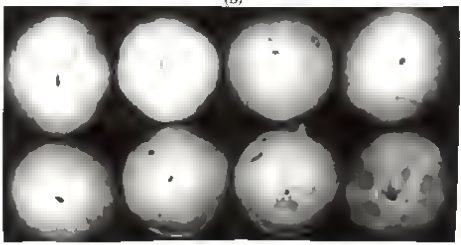
Figure 3-14 Temporal relationship between detectable enzyme activities of PG □ , PME ■ , Beta-galactosidase _ , and firmness ■ of pericarp discs of pink tomato fruit following irradiation at 0 (A), 0 72 ± 0 11 (B) and 1 41 ± 0 15 kGy (C), and storage at 20°C for 7 days. Enzyme activity was expressed as µmol per gram fresh weight per min. Higher Newtons value indicate softer tissue

Fig. 3-15 Color development after seven days following irradiation of mature-tomato fruit irradiated at 0 (control, A), 0.73 ± 0.11 kGy (low dose, B), c 2.21 ± 0.22 kGy (high dose, C) and storage at 20°C

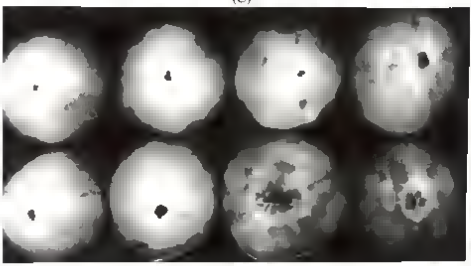
(A)



(B)



(C)



CHAPTER 4
MODIFICATION OF CELL WALL MATRIX POLYMERS INDUCED BY IONIZING IRRADIATION

Introduction

Considerable research has been directed toward understanding the mechanism of the textural changes associated with normal ripening of fruits, focusing primarily on the modifications occurring in the composition of cell wall components. Characteristics associated with normal ripening of fruit that account for tissue softening include solubilization and degradation of pectic polysaccharides, and losses of the cell wall neutral sugars galactose and arabinose. These changes have been reported for tomato (Wallner and Bloom, 1977, Gross and Wallner, 1979, Gross, 1984) as well as many other fruits (Labavitch, 1981, Gross and Sams, 1984).

Softening, in addition to its induction as a process associated with normal ripening, can also be induced by ionizing irradiation. The potential application of irradiation as a postharvest technique is sometimes limited by excessive tissue softening (Abdel-Kader et al., 1968a, Maxie and Sommer, 1963, Cooper and Salunkhe, 1963, Skou, 1963, Ahmed et al., 1972, Brecht et al., 1992, d'Amour et al., 1993). Solubilization of pectic polysaccharides and the release of uronic acid (Kertesz et al., 1956, McArdle and Nehemias, 1956, Boyle et al., 1957, Kertesz et al., 1964, Somogyi and Romani, 1964, Yasir et al., 1987, d'Amour et al., 1993) associated with the

hydrolysis of polysaccharides to lower molecular weight fragments (Maxie and Kader, 1966) have been considered the major events that characterize irradiation-induced softening. While some work has suggested that irradiation induces a decrease in total pectin levels, as in irradiated strawberry (d'Amour et al , 1993), others have reported that the total amount of pectic substances were unaffected in irradiated cucumber pickles (Howard and Buescher, 1989), or increased in irradiated strawberry fruit (Bellì Donnini and Stornaiuolo, 1969) Neutral sugars, mainly arabinose and galactose, which present as major components of pectic polymers (Darvill and McNeil, 1980) were reported to be unaffected or subject to minor changes by irradiation (Howard and Buescher, 1989, d'Amour et al 1993) Levels of proteins in irradiated tomato fruit have been observed to decrease immediately following irradiation followed by a general recovery within 24 hr (Ferullo et al , 1994, Triantaphylides et al , 1994)

The objective of the present study was to investigate the modifications occurring in the cell wall matrix polysaccharides of tomato fruit in response to ionizing irradiation Particular emphasis was placed on the influence of irradiation on the structural properties of cell wall pectin, including associated neutral sugars and on salt-extractable cell wall proteins

Materials and Methods

Plant Materials

Tomato fruit (*Lycopersicon esculentum* L. cv Sunny) at the mature-green and pink stages of development were obtained and treated as described in Chapter 3

In a separate experiment, tomato fruit (Flavr Savr PG antisense-CR633g) at the mature-green stage of development were harvested from plants grown at the IFAS Horticultural Farm near Gainesville FL. Fruit were selected for uniformity of size and freedom from defects, surface sterilized with 1 mM sodium hypochlorite, rinsed and dried at 23°C (±1) Fruit were then subjected to irradiation as described below

Irradiation Treatment

Irradiation of mature-green and pink tomato fruit was performed as described in Chapter 3 Irradiated fruit were placed in crushed ice immediately following irradiation to arrest compensatory responses (Romani, 1984) that might mask the effects of irradiation

Preparation of Alcohol-Insoluble Solids

Tomato fruit were cut radially the gel and placental tissue removed, and 100 g of pericarp tissue were then homogenized in 400 ml of 100% cold ethanol (final ethanol concentration of 80%) using a Polytron (Brinkmann Instruments, Westbury, N Y) set at speed # 7 for 3 minutes The same procedures were repeated in the PG antisense 'Flavr Savr' tomato fruit and both pericarp and gel tissues were prepared The alcohol-insoluble solids (AIS) were prepared as described by (Huber 1991) with slight modifications (Huber and O'Donoghue, 1993) One-hundred ml of the EtOH suspensions (homogenized pericarp tissue in 80% EtOH) were filtered through Miracloth (Calbiochem Corp, La Jolla, CA) in a Buchner funnel under aspiration and washed with 100 ml of 80% EtOH The residue was recovered from the filter paper and transferred to 45 ml Tris-buffered phenol, pH 7 0 (Huber 1992) for 30 min at 23°C (±1) while stirring at

low speed. The suspension was readjusted to 80% EtOH and held at 20°C for 30 min to facilitate the re-precipitation of the AIS. After another filtration through Miracloth and washing with 200 ml 80% EtOH, the residue was transferred to 100 ml chloroform methanol (1 1 v/v) for 30 min at 23°C (±1) while stirring at low speed. The suspension was then filtered, washed with 200 ml acetone, partially dried under aspiration, and transferred into a glass petri dish and placed in an oven at 34°C for 12 hr. The AIS preparations were stored in a desiccator at 23°C (±1) until needed.

Uronic Acid Analysis

Total uronic acid content in the AIS was determined as described by Ahmed and Labavitch (1977). Approximately 7 mg of AIS powder in 2.5 ml of ice cold, concentrated sulfuric acid (H_2SO_4) were incubated for 5 min in an ice bath. One ml of cold di H_2O was added drop-wise while stirring and repeated within 5-10 min. After a 10-min interval following the final addition of di H_2O, the solution was brought to a volume of 50 ml with cold di H_2O. The suspension was filtered (GF/C) and total uronic acid in the filtrates was determined colorimetrically using the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Hanson, 1973).

Isolation and Purification of Soluble Polyuronides

Preparation of H_2O-, chelator [1,2-cyclohexylenedinitrilotetraacetic acid (CDTA)]-, and alkali [sodium carbonate (Na_2CO_3)]-soluble pectic polymers was determined as described by Jarvis (1982). Approximately 30 mg of AIS were incubated in 14 ml di H_2O and placed on a shaker for 6 hr at 23°C (±1). After filtration and washing, the residue was transferred to 14 ml 50 mM sodium acetate (NaOAc), 50 mM CDTA, pH 6.5 for 6 h at

23°C (±1). The residue after filtration was transferred to 14 ml of 50 mM Na_2CO_3 for 6 hr at 23°C (±1). The suspensions were filtered and uronic acid in the filtrates determined colorimetrically (Blumenkrantz and Asboe-Hanson 1973) Soluble pectic polymers were subjected to size exclusion chromatography as described below

Gel Chromatography

Gel filtration and molecular mass properties of H_2O-, CDTA-, and Na_2CO_3-soluble polyuronides were determined on a bed (31 cm high, 1.5 cm wide) of Sepharose CL-2B-300 Approximately 0.5 mg polyuronide (galacturonic acid equivalents) were applied to the column and eluted with 200 mM ammonium acetate (NH4CH3CO) at pH 5 0 (Mort et al., 1991) Two-ml fractions were collected at a flow rate of 60 ml hr^{-1} Aliquots (0.5 ml) were analyzed for uronic acid content as described above The column void (v_o) and total (v_t) volumes were identified by the elution positions of Dextran (2000 kDa) and glucose, respectively

Compositional Analysis of Pectin-Associated Neutral Sugars

Neutral sugar analysis of the soluble polyuronide fractions (H_2O-, CDTA-, and Na_2CO_3-soluble) was carried out as described by Blakeney et al. (1983), with some modification Prior to analysis, pectic polymers were processed to remove nonstructurally associated neutral sugars Samples of CDTA-, and Na_2CO_3- soluble polyuronides were dialyzed (SpectraPor 2,000 MWCO) against di H_2O at 5°C for 24 hr with three changes After dialysis, the samples were applied to a bed (15 cm high, 2 5 cm wide) of DEAE-Sephadex (A 25-120, Sigma) equilibrated in 4 mM sodium phosphate (NaPO4, pH 6 8) After sample application, the phosphate buffer was passed through the

column to elute free neutral sugars, after which the column was step eluted with 600 mM NaCl to remove polyuronides. Salt-eluted fractions containing carbohydrate (phenol-sulfuric acid test) were collected and 0.5 ml was used to determine total uronic acids. Samples were then concentrated, and 0.5 to 1 mg galacturonic acid equivalents, along with 200 µg myoinositol (as an internal standard for GC analysis) were added to vials (Pierce Reacti-Vials), and placed on a Multi-Heater Block (Lab-line Instruments, Inc.) at 45°C under a filtered air stream. After drying, 1 ml of 2 N trifluoroacetic acid (TFA) was added, and the samples incubated at 120°C for 1 hr. After hydrolysis, the vials were opened and the samples dried at 45°C under a filtered air stream. The samples were then washed twice with di H_2O and left to dry each time on a heater block at 45°C with air flow. Afterward, 100 µl of 3 M ammonium hydroxide (NH_4OH) and 1 ml sodium borohydride-dimethyl sulfoxide ($NaBH_4$-DMSO [2 g/100 ml]) were added to the hydrolyzed samples and then incubated for 90 min at 40°C. One hundred µl of 18 M acetic acid, 200 µl of 1-methylimidazole ($C_4H_6N_2$), and 2 ml of acetic anhydride ($[CH_3CO]_2O$) were added, and the samples were incubated for 10 min at 23°C (±1). Five ml of di H_2O, and 1 ml of dichloromethane (CH_2Cl_2) were added and the bottom phase was collected in a microcentrifuge tube. One ml di H_2O was added, and the samples were then centrifuged and the bottom phase collected. The last step was repeated twice and the bottom phase (the alditol acetate in dichloromethane) used for GC analysis. Gas chromatography (Hewlett Packard, Model 5710A, Atlanta, GA) on a column (HP-5 [5% phenyl methyl silicone, 25 m× 0.2 mm× 0.33 µm film thickness) operated at an initial

temperature of 210°C for 5 min and increased to 230°C at a rate of 4°C per min with flame ionization detector, was used for the neutral sugar determination

Hemicellulose Preparations

AIS preparations treated with Tris-buffered phenol (Huber 1992) were used as starting materials for hemicellulose preparation Isolation and preparation of hemicellulose was carried out using the Carpita (1984) method, with slight modification Samples of 600 mg each of AIS derived from mature-green and pink tomato fruit irradiated at 0, 0 73 ± 0 11, or 2 21 ± 0 22 kGy were incubated in 60 ml of 90% dimethyl sulfoxide (DMSO) for 16 hr at 23°C (± 1) while stirring (for starch removal) After filtration through Miracloth, the residues were transferred to 60 ml of 20 mM sodium acetate (NaOAc), 10 mM disodium ethylenediamine tetraacetate (EDTA), pH 5 0, for 4-6 hr at 23°C (± 1) to remove some of the pectic polysaccharides The samples were then centrifuged for 20 min at 10 000 × g, the pellets washed with di H_2O, and transferred to 10 ml of 4 M potassium hydroxide (KOH) containing 3 mg ml^{-1} sodium borohydride ($NaBH_4$), and incubated at 23°C (± 1) overnight After filtration through Miracloth, the residues were washed with 10 ml of KOH including 3 mg ml^{-1} $NaBH_4$, filtered again, and the supernatants combined and neutralized with acetic acid The neutralized samples containing hemicellulose were then dialyzed (2,000 MWCO) for 3 days (with three changes every day) as follows 24 hr in running tap H_2O, 24 hr in 10% methanol (MeOH) at 5°C, and 24 hr in di H_2O After dialysis, the concentrated samples containing 1 5 mg hemicellulose were used for chromatography as described above, except that Sepharose CL-2B-200 column was used.

Protein Preparations

Preparation of salt extractable proteins from mature-green and pink tomato fruit irradiated at 0, 0 72 ± 0 11, or 1 43 ± 0 15 kGy was carried out as described in Chapter 3

Electrophoresis of Tomato Fruit Proteins

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was carried out using the modified method described by Schagger and von Jagow (1987) In this method, Tris-tricine is used as the electrode buffer instead of Tris-glycine used in Laemmli gel method (Schagger and von Jagow, 1987) The gel apparatus (Mini-Protean II Electrophoresis Cell, BIO-RAD) consisted of two buffer reservoirs separated by a gel sandwiched between two glass plates. A template "comb" was used to form the wells by placing it within the stacking gel In this apparatus, the anode is on the top (or inner chamber) and the cathode electrode is at the bottom (or outside chamber) The separating gel was prepared by mixing 6 7 ml of gel buffer (3 M Tris, pH 8 45), 6 7 ml of 30% acrylamide, pH >6 0, 3 1 ml of 2% bis-acrylamide (w/v) pH > 6 0, 0 1 ml of 20% SDS (w/v) and 3 4 ml di H_2O After de-gassing the mixture, 0 5 µl of N,N,N',N', tetramethylethylenediamine (TEMED) and 50 µl of 10% ammonium persulfate (APS) were added to 12 ml of the separating gel The solution was pipetted into the gel space (in between the glass plates) using a Pasteur pipet One-half ml of butanol (pre-saturated with H_2O) was overlayed on the unpolymerized gel, and the gel was allowed to polymerize for 30 min The stacking gel was prepared using 1.24 ml of gel buffer, 0 5 ml of 30% acrylamide, 0 24 ml of 2% Bis (w/v), 25 µl of 20% SDS (w/v), and 3 ml di H_2O Butanol was removed after the separating gel became polymerized, the comb was inserted

halfway, and 4-5 ml of the stacking gel (containing 0 5 µl TEMED and 50 µl of 10% APS) were added and the comb was inserted all the way The stacking gel was then allowed to polymerize for 30 min, after which the comb was removed slowly and the cathode buffer [prepared in advance from 1 M Tris, pH 8 25, 1 M tricine, 1% SDS(w/v)] was added to the reservoir until the wells were filled Anode buffer (24 2 g of 2 M Tris pH 8 9, and 85 8 ml di H_2O) was poured into the gel tank, and 10 µl from the protein standard and each protein sample were then added to the wells The protein samples were prepared for electrophoresis as follows 120 µl of trichloro-acetic acid (TCA) were added to 1 ml of each sample containing the protein, and then incubated for a minimum of 30 min in the refrigerator Afterward the sample was centrifuged for 5 min at 15,000 × g (Biofuge 15 Heraeus Instruments), and the supernatant was discarded. The pellet containing the precipitated protein was washed 2-3 times with cold methanol, and 20 µl of Laemmeli gel [62 5 mM Tris, pH 6 8, 2% SDS (w/v), 720 mM 2 mercaptoethanol, 2 ml glycerol 0 1% bromophenol blue (w/v)] were added and the mixture was vortexed and heated for 5 min at 85°C in a Multi-Heater Block

After electrophoresis for approximately 1 hr (15-20 min at 50 v and 40-45 min at 125 v), the gel was removed, placed in a 140 mm petri dish containing 20 ml Coomassie blue gel stain [0 2 g of Coomassie blue R-250 (w/v), 400 ml of methanol (w/v), 550 ml di H_2O, 50 ml of glacial acetic acid (w/v)], and agitated for 30 min at 23°C (± 1) on a shaker Afterward the stain was removed, and 50 ml of Coomassie gel de-stain consisting of 500 ml of methanol, 100 ml of glacial acetic acid, and 850 ml of di H_2O, were added along with a piece of foam (to help absorb the Coomassie stain, which diffuses out of the

gel) and placed on a shaker overnight The gal was then removed, rinsed with di H_2O containing 1% v/v glycerol, dried using a Plexiglass frame and two pieces of BioGel membrane, and photographed.

Protein molecular mass markers (all from Promega) were phosphorylase B (97,400 D), glutamate dehydrogenase (66 200 D), ovalbumin (42,700 D), Aldolase (40,000 D), carbonic anhydrase (31,000 D), soybean trypsin inhibitor (21,500 D), and lysozyme (14,400 D)

Statistical Analysis

Data from the three irradiation treatments were analyzed as a completely randomized design with three replicates Data obtained from each fruit maturity stage were analyzed separately using Analysis of Variance (ANOVA), and means were separated by the Least Significant Difference method at the 0 05 level

Results and Discussion

Total Uronic Acid Content and Polyuronide Solubility

In view of the marked effect of irradiation on fruit and pericarp firmness shown in Chapter 3 (Figs 3-1 to 3-6), measurements of the total uronic acid content and polyuronide solubility were employed to study the impact of irradiation on the structural features of cell wall pectic polymers Alcohol insoluble solids (AIS) derived from 'Sunny' tomato pericarp were used for these measurements Two separate experiments were conducted measuring total uronic acid content and solubility and results are shown in Tables 4-1 and 4-2

Total uronic acid content in AIS derived from pericarp of mature-green and pink fruit was unaffected by the low irradiation treatment compared to the total uronic acid content of control fruit (Table 4-1) However, decreases in the total uronic acid content of 11% and 22% were observed in mature-green and pink fruit, respectively, irradiated at the higher dose compared the control treatments (Table 4-1) In the second experiment, no significant changes were noted at either dose for mature-green or pink fruit compared to the control (Table 4-2) It is clear that the decrease in total uronic acid was more pronounced at the highest irradiation dose (2.21 ± 0 22 kGy), which was used in the first experiment (Table 4-1), compared to the other, lower irradiation doses used (Table 4-1 and 4-2)

The decrease in total uronic acids possibly resulted from a direct effect of irradiation on the pectic polymers. Similar effects on the total uronic acids for AIS derived from apples and carrots irradiated at 2 5 kGy (McArdle and Nehemias 1956), peaches and pears irradiated at 3 0 to 9 0 kGy (Somogyi and Romani, 1964), and apples, carrots and beets irradiated at 4 0 kGy (Kertesz et al., 1964) were observed. In strawberry fruit, a decrease in galacturonate from 59% to 52% of the total cell wall carbohydrates was noted in response to irradiation at 4 0 kGy (d'Amour et al., 1993) On the other hand, no change in total pectic substances (uronic acid assay) was observed in AIS derived from pickling cucumber irradiated at 0 5 or 1 0 kGy (Howard and Buescher 1989) and in ripe strawberry fruit irradiated at 2 0 kGy (Belli-Donnini and Stomaiuolo, 1969) In the present study, the reduction in total uronic acid content of AIS preparation

indicated pectin degradation occurred, with the production of products that were washed out as ethanol-soluble fragments during the extraction procedure

Huber (1983b) stated that the most frequently reported event during normal fruit ripening is the increase in solubility of pectins, even though an overall loss of pectins has also been observed. In this study, the changes in the total uronic acids of mature-green fruit were associated with increases in H_2O-, and CDTA-soluble polyuronides, with slight or no changes in the Na_2CO_3-soluble polyuronides (Table 4-1 and 4-2)

A nearly 2-fold increase in the H_2O-soluble polyuronides was observed in extracts derived from irradiated mature-green fruit compared to the H_2O-soluble polyuronides from mature-green control fruit (Table 4-1 and 4-2) No significant differences were observed in the uronic acid content of H_2O-soluble fractions of pink fruit irradiated at the lower dosage (Table 4-1, and 4-2). However, considering the lower amount of total uronic acid in the high dose treatments, and the proportionally greater amount of H_2O-soluble polyuronide in these treatments, there appears to be a slight increase in the H_2O-soluble uronic acids in AIS from pink fruit irradiated at the higher dose compared to the control (Table 4-1 and 4-2) Levels of CDTA-soluble polyuronides in AIS derived from irradiated mature-green fruit increased relative to the control, although this increase was not as dramatic as that noted for the H_2O-soluble polyuronides (Table 4-1 and 4-2) No changes were noted in the CDTA-soluble polyuronides in irradiated pink fruit compared to the control However, considering the lower amount (6%) in total uronic acid in the high dosage treated pink fruit relative to the control, and the proportionally greater amount of the soluble fraction to the total uronic acid, a 5% increase in CDTA-soluble

polyuronides appears to have occurred (Table 4-2) These results demonstrate that the solubility properties of pectins are influenced by irradiation, consistent with earlier studies of irradiation effects on strawberry (d'Amour et al , 1993), and pear and peach

Table 4-1 Total uronic acid content, H_2O-, CDTA-, and Na_2CO_3-soluble polyuronides[1] in AIS derived from mature-green and pink fruits following irradiation at 0 (control), 0 73 ± 0 11 (low dose), or 2.21 ± 0 22 kGy (high dose)

Treatment	Total uronic acid	Soluble polyuronides		
		H_2O-	CDTA-	Na_2CO_3-
Mature green				
Control	313.5a[2]	28 8b	35 4b	87 0a
Low dose	311 3a,b	52 6a	43 4a	85 0a
High dose	279 7b	44 7a	43 0a	72 0a
Pink				
Control	337 5a	47 9a	38 0a	76 0a
Low dose	337 4a	57 5a	42 3a	67 0a
High dose	263 0b	47 6a	40 0a	65 0a

[1] Means are the average of three separate extractions, and are expressed as µg uronic acid per mg powder

[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0 05 level by LSD method.

(Somogyi and Romani, 1964) fruits On the other hand, these results are in contrast to previous reports that postulated that no changes were observed in the H_2O-soluble uronic

acids in irradiated ripe strawberry (Belli-Donnini and Stornaiuolo, 1969) and tomato (Yasir et al., 1987) fruits

Table 4-2 Total uronic acid content, H_2O-, CDTA-, and Na_2CO_3-soluble polyuronides[1] in AIS derived from mature-green and pink tomato fruits following irradiation at 0 (control), 0.72 ± 0.11 (low dose), or 1.41 ± 0.15 kGy (high dose)

Treatment	Total uronic acid	Soluble polyuronides		
		H_2O-	CDTA-	Na_2CO_3-
Mature-green				
Control	312.5a[2]	23.3b	34.0b	82.0a
Low dose	309.0a	49.0a	39.7a,b	78.7a,b
High dose	310.0a	48.7a	40.7a	68.0b
Pink				
Control	343.3a	45.0a	37.7a	74.3a
Low dose	327.7a	58.7a	40.0a	65.3a
High dose	323.0a	55.7a	40.0a	68.7a

[1] Means are the average of three separate extractions , and are expressed as µgs uronic acid per mg powder

[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0.05 level by LSD method

No changes were observed in the Na_2CO_3-soluble polyuronides in the AIS of irradiated mature-green and pink tomato fruit relative to the controls (Table 4-1 and 4-2), except significantly lower levels of Na_2CO_3-soluble polyuronides in the high irradiation

dose treatment for mature-green fruit compared to the control fruit in the second experiment (Table 4-2) These results are in agreement with earlier reports for irradiated ripe strawberry fruit (Belli-Donnini and Stornaiuolo, 1969), and in contrast to earlier reports for irradiated tomato fruit (McArdle and Nehemias 1956, Kertesz et al 1964, Yasir et al , 1987).

A comparison of the magnitude of the increase in the H_2O-soluble polyuronides to that of the CDTA-soluble polyuronides in irradiated mature green and pink fruit revealed a higher magnitude in the former These results were obtained with sequential extraction of AIS in H_2O followed by CDTA. This approach has been employed by many researchers to study the polyuronide changes during development The changes observed in pectin solubility between irradiated and non-irradiated mature-green fruit (that were uniformly selected at the same stage of development at the time of irradiation and placed in crushed ice immediately following irradiation until processed) were very dramatic suggesting that the effect of irradiation is direct In such a case, the possibility exists that the degraded polyuronides might have been easily extracted by any extractant regardless of the characteristics of the different extractants

To investigate this possibility, a reversed extraction sequence (i.e CDTA followed by di H_2O) of uronic acid extraction was used (Table 4-3) The CDTA-soluble polyuronides increased in both irradiated mature-green and pink fruit relative to the controls (Table 4-3), whereas the H_2O-soluble polyuronides were unaffected by irradiation in mature-green fruit, and decreased in pink fruit compared to the controls

Table 4-3 Total uronic acid content, CDTA-, and H_2O-soluble polyuronides[1] in AIS derived from mature-green and pink tomato fruits following irradiation at 0 (control) 0.72 ± 0.11(low dose), 1.41 ± 0.15 kGy (high dose), or 2.21 ± 0.22 kGy (very high dose)

Treatment	Total uronic acid	Soluble polyuronides	
		CDTA-	H_2O-
Mature-green			
Control	311.5a[2]	47.0b	49.2a
Low dose	313.3a	53.0b	48.6a
High dose	310.0a	64.0a	53.3a
Very high dose	279.7b	72.2a	52.1a
Pink			
Control	337.5a	56.7b	62.2a
Low dose	337.4a	60.2b	58.9b
High dose	332.0a	68.2a	44.1b
Very high dose	263.0b	75.9a	37.5c

[1] Means are the average of three separate extractions and are expressed as µg uronic acid per mg powder

[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0.05 level by LSD method

(Table 4-3). Interestingly, the increases in the CDTA-soluble polyuronides from irradiated fruit at both maturity stages were noted to be higher than those when the typical sequence (H_2O followed by CDTA) was used (Tables 4-1 and 4-2). Unlike those obtained with mature-green fruit (Tables 4-1, and 4-2), no significant differences were

noted in the H_2O-soluble polyuronides from irradiated mature-green fruit (Table 4-3) However a decrease was noted in the H_2O soluble polyuronides derived from pink fruit compared to those derived from the control fruit, and that decrease was dosage dependent These observations indicate that the effect of irradiation was a direct one, triggering a breakdown in the structure of the polyuronide chains, presumably resulting in the solubillzation of the polyuronides to such an extent that they can be easily extracted by any extractant that is used first

To confirm that the decrease in total uronic acid and increase in soluble polyuronides were directly related to the effect of irradiation rather than to enzymic reactions, an additional experiment employing PG antisense tomato fruit was conducted In addition, since pericarp tissue was apparently more affected by irradiation than the whole intact fruit, as evident by the excessive softening observed in pericarp discs compared to the intact fruit (Chapter 3, Figs 3-1 to 3-6), a comparative analysis was made between pericarp and gel tissues PG antisense tomato fruit that contain only trace amounts of PG enzyme and soften very slowly were subjected to high doses of irradiation (0 or 2 675 ± 0 075 kGy) to determine the effects of irradiation in the absence of PG action

Total uronic acid content was not affected by irradiation in either tissue (Table 4-4) However, total uronic acid was quantitatively twice as high in the pericarp tissue as in the gel tissue, an observation that has been reported in the normal tomato fruit (Huber and Lee, 1986). It was noted that CDTA-soluble polyuronides increased by

46% and 39% in the irradiated pericarp and gel tissue respectively However the proportionally higher total uronic acid present in pericarp tissue suggests that it has a more significant role in the fruit softening than the gel tissue. In addition, the absence of

Table 4-4 Total uronic acid content and CDTA-soluble polyuronides[1] in AIS derived from homogenates of pericarp or gel tissue of PG antisense tomato fruit irradiated with gamma rays at 0 or 2 675 ± 0 075 kGy

Treatment	Total uronic acid	CDTA-Soluble polyuronides
Pericarp tissue		
Non-irradiated.	311 6a[2]	80 7b
Irradiated	307 8a	118 0a
Gel tissue		
Non-irradiated	127 6a	37 3b
Irradiated	145 6a	51 8a

[1] µgs uronic acid / mg powder
[2] Values in columns for each type tissue followed by the same letter within are not significantly different at the 0 05 level by LSD method

PG enzyme in the antisense tomato would suggest that the effect of irradiation on uronic acid solubility was a direct effect rather than mediated by enzymes

Molecular Weight Analysis of Soluble Polyuronides

In previous studies of the solubility and M, changes in specific pectic fractions of irradiated fruits (Somogyi and Romani 1964 d'Amour et al , 1993), the procedures for

isolation of pectic polysaccharides involved exposure to potentially degradative conditions (high temperatures and/or acid). In other cases (Yasar et al., 1987), there were no indications that attempts were made to inactivate endogenous pectin-depolymerizing enzymes. Consequently, it is difficult to attribute the observed changes in pectin properties to the effects of irradiation vs thermal or enzymic degradation unrelated to the irradiation treatments. In the present study, tomato fruit were placed in crushed ice immediately following irradiation, quickly processed into EtOH homogenates and exposed to Tris-buffered phenol (Huber, 1992). This solvent has no detectable effects on pectin solubility properties and cell wall calcium levels (Huber, 1991), and is apparently completely effective at inactivating pectin depolymerases and other hydrolases. A detailed description of the methods of preparation of pectic fractions for gel filtration, chromatographic conditions, and the specific matrix (Sepharose-2B-300) employed for gel chromatography can be found in previous reports (Huber, 1991, 1992; Huber and O'Donoghue, 1993). The gel filtration matrix employed is an intermediate-resolution gel with an exclusion limit of 20×10^6 Da for polyuronides. This gel is also capable of providing reproducible fractionation patterns for the relatively polydisperse tomato pectins at all stages of development (Huber, 1992). The elution buffer used (200 mM NH_4CH_3CO, pH 5.0) has been shown to minimize the intermolecular aggregation of the highly anionic pectin polymers, and to yield more reproducible elution profiles (Mort et al., 1991).

Sepharose CL-2B-300 analysis of soluble polyuronides from irradiated mature green and pink tomato fruit (Figs. 4-1 to 4-6) were performed on pectins solubilized from

AIS incubated in di H_2O followed by incubation in CDTA (e.g. Tables 4-1 and 4-2). Irradiation was accompanied by significant downshifts in the M_r of H_2O-soluble polyuronides from mature-green tomato fruit (Figs. 4-1 and 4-2 A, B, and C). The effect was dosage dependent, as shown by the more extensive size downshifting in the high irradiation treatments (1.41 ± 0.15 and 2.21 ± 0.22 kGy) vs the low irradiation treatments (0.72 ± 0.11 and 0.73 ± 0.11 kGy). Although pink fruit were characterized by an inherently lower M_r of soluble polyuronides (Figs. 4-3 and 4-4, A) compared to mature-green fruit, a consequence of ripening-related pectin metabolism (Huber and O'Donoghue 1993), more extensive, dosage-dependent depolymerization was noted in response to irradiation (Figs. 4-3 and 4-4, B and C).

The CDTA-soluble polyuronides were of lower M_r than the H_2O-soluble polyuronides, with a high proportion of the polymers eluting at or near the void volume of Sepharose CL-2B-300. Even so, the CDTA-soluble pectins were dramatically affected by irradiation (Figs. 4-5 and 4-6 A, B, and C). A dramatic downshift in pectin M_r was noted for fruit of both maturity classes. As observed in the H_2O-soluble polyuronides, the downshift in the M_r of the CDTA-soluble polyuronides was also dosage dependent (Figs. 4-5, and 4-6 A, B and C).

Sepharose CL-2B-300 profiles of the H_2O-, and CDTA-soluble polyuronides at both maturity stages demonstrate clearly the downshifting of the M_r of these polymers in irradiated fruit. The downshifting of the M_r indicate that extensive depolymerization had occurred in polyuronides from irradiated fruit. Since all the fruits were held in ice immediately following irradiation until AIS preparation (within 4-5 hr following

irradiation) to arrest postirradiation compensatory response (Romani, 1984), and the employment of Tris-buffered phenol to inactivate pectin depolymerase (Huber, 1992) the extensive depolymerization was presumably a direct effect of irradiation In addition, the extensive solubilization of the H_2O-, and CDTA soluble polyuronides in the irradiated fruit (Tables 4-1 and 4-2), leads to the conclusion that the mechanism by which irradiation affects the pectic polymers is direct, occurring via solubilization as well as depolymerization

Sepharose CL-2B-300 profiles of the CDTA-soluble polyuronides from AfS derived from the pericarp of PG antisense tomato fruit irradiated at 2.675 ± 0.075 kGy showed a slight downshifting in M compared to the control (Fig 4-7) Similarly, a downshifting in the M, of CDTA-soluble polyuronides from the gel tissue occurred compared to the control (Fig 4-8)

Effect of Irradiation on Hemicellulose

Sepharose CL-4B-200 profiles of hemicelluloses from irradiated mature-green and pink fruit are shown in Figures 4-9 and 4-10, A, B, C As these figures illustrate there was no apparent change in the high M, polymers in irradiated fruit compared to the control A very slight increase was noted in the smaller hemicellulose polymers in irradiated fruit compared to the control at both maturity stages

Ripening-associated modifications of hemicellulose (alkali-soluble wall polymers) derived from tomato fruit include a progressive loss in high M, ($\geq 3.5 \times 10^5$) polymers and an increase in low M, ($< 4 \times 10^3$) polymers (Huber, 1983a) Surprisingly, only slight changes have been noted in the quantity of hemicelluloses, even though gel filtration

chromatography indicated a dramatic decrease in the size of these polymers during ripening of hot pepper (Gross et al., 1986), strawberry (Huber, 1984) and muskmelon (McCollum et al , 1989) fruits Since hemicellulose is considered a collection of diverse polymers efforts have been made to identify the specific polysaccharide component(s) that might be responsible for the shift in molecular mass profiles Tong and Gross (1988) identified two hemicellulosic fractions in tomato fruit, HFI and HFII, extractable in 4M and 8M KOH, respectively Of these, only HFII showed a proportional increase in low molecular mass polymers during ripening Furthermore the shifting in hemicellulose elution behavior might involve synthesis of low molecular mass polymers enriched in mannosyl and glycosyl residues, possibly reproducing glucomannans (Gross, 1988 Tong and Gross 1988)

In the present study, no loss of high M, polymers and the appearance of smaller polymers in very low quantities in irradiated fruit were noted. This indicates that the effect of irradiation on hemicellulosic polymers is negligible On the other hand the lack of appearance of the smaller polymers in quantities that indicate a pronounced effect may be due to the fact that 4 M KOH was used to extract the hemicelluloses, which provides HFI fractions only (Tong and Gross, 1988) This might explain why only a slight impact of irradiation on hemicelluloses was noted Since the effect of irradiation on hemicelluloses was negligible no further investigation were performed on these

Effect of Irradiation on Pectin-Associated Neutral Sugars

The AIS preparations used for the neutral sugar analysis were derived from mature-green and pink fruit irradiated at 0, 0 72 ± 0 11 or 1 41 ± 0 15 kGy and held in ice

immediately following irradiation until processing. In general the neutral sugars in the H_2O-, CDTA-, and Na_2CO_3-soluble polyuronides from irradiated fruit at both maturity stages were unaffected or only slightly affected by irradiation. An effect on neutral sugars was noted in only two cases for mature-green fruit irradiated at the higher irradiation dose (Tables 4-5 and 4-9). A decrease of 7% was noted in the total neutral sugar content of the H_2O-soluble polyuronides from mature-green fruit at the high irradiation dose treatment relative to the control fruit (Table 4-5). This decrease was reflected by a proportionally lower level of galactose in the H_2O-soluble polyuronides in mature-green fruit treated at the high irradiation dose compared to the control (Table 4-5). No other significant differences between the low dose and control treatments were noted in the neutral sugars in the H_2O-soluble polyuronides (Table 4-5). Neither were significant differences noted in the total or individual neutral sugars in the H_2O-soluble polyuronides of irradiated pink fruit (Table 4-6).

As with the H_2O-soluble polyuronides, no significant differences between irradiated and control fruit at both maturity stages were noted in total or in individual neutral sugars in the CDTA-soluble polyuronides (Tables 4-7 and 4-8). Similarly, no significant differences in total neutral sugars were noted in Na_2CO_3-soluble polyuronides (Tables 4-9 and 4-10). However, a significant increase in glucose and a significant decrease in galactose were detected in the Na_2CO_3 soluble polyuronides from the high irradiation treated mature-green fruit compared to the control fruit (Table 4-9). The

Table 4-5 Compositional analysis of neutral sugars[1] in the H_2O-soluble polyuronides[2] derived from mature-green tomato fruit[3] following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose)

Treatment	Control	Low dose	High dose
Rhamnose	5.5a[4]	6 5a	6 9a
Arabinose	17 2a	18 2a	18 4a
Xylose	5.3a	6 4a	6 3a
Mannose	7 0a	8 0a	7 8a
Glucose	10 0a	12.3a	12 8a
Galactose	55.5a	49 6a,b	47 7b
Neutral sugar : uronic acid	0 140a	0 140a	0 130b

[1] Means are the average of three separate derivatizations, and are expressed as mole % individual neutral sugar to total neutral sugars.

[2] H_2O-soluble polyuronides were extracted by incubation of 30 mg of AIS derived from irradiated mature-green fruit in 14 ml of dh H_2O for 6 hr at 23°C (±1)

[3] Following irradiation, fruit were placed immediately in crushed ice until processed

[4] Values within a row followed by the same letter are not significantly different at the 0 05 level by LSD method

increased quantity of glucose apparently offset the decrease in galactose, negating overall changes in total neutral sugars No differences among the treatments were noted in either total or individual neutral sugars of the Na_2CO_3-soluble polyuronides from pink fruit (Table 4-10)

Table 4-6 Compositional analysis of neutral sugars[1] in the H_2O-soluble polyuronides[2] derived from pink tomato fruit[3] following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose) [1]

Treatment	Control	Low dose	High dose
Rhamnose	6 9a[4]	5 8a	4 8a
Arabinose	17 0a	18 9a	18 7a
Xylose	7 0a	7 7a	9 3a
Mannose	8 5a	7 2a	7 8a
Glucose	10 8a	12 8a	13 0a
Galactose	49 8a	47 6a	46 4a
Neutral sugar uronic acid	0 120a	0 120a	0 116a

[1] Means are the average of three separate derivatizations, and are expressed as mole % individual neutral sugar to total neutral sugars.

[2] H_2O-soluble polyuronides were extracted by incubation of 30 mg of AIS derived from irradiated pink fruit in 14 ml of di H_2O for 6 hr at 23°C (±1)

[3] Following irradiation, fruit were placed immediately in crushed ice until processed

[4] Values within a row followed by the same letter are not significantly different at the 0 05 level by LSD method

Generally, the levels of total neutral sugars associated with pectic polymers were in the order H_2O-, CDTA-, and Na_2CO_3-soluble, with the H_2O-soluble fraction containing the highest proportion. Higher levels of neutral sugars were observed in green fruit compared to pink fruit for all treatments Previous studies of the effect of irradiation on the composition of the neutral sugars (pectin-associated or total cell wall neutral sugars) reported that neutral sugars were either unaffected or subject to minor changes as a result

Table 4-7 Compositional analysis of neutral sugars[1] in the CDTA-soluble polyuronides[2] derived from mature-green tomato fruit[3] following irradiation at 0 (control), 0 72 ± 0 11 (low dose), or 1 41 ± 0.15 kGy (high dose)

Treatment	Control	Low Dose	High Dose
Rhamnose	5 8a[4]	5 9a	5 7a
arabinose	19 6a	20 5a	20.5a
Xylose	7 4a	6 7a	7 4a
Mannose	2 5a	2 4a	3 0a
Glucose	6.7a	6 9a	7 5a
Galactose	58 0a	57 6a	55 9a
Neutral sugar uronic acid	0 120a	0 116a	0 115a

[1] Means are the average of three separate derivatizations, and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] CDTA-soluble polyuronides were extracted by incubating of 30 mg of AIS derived from irradiated mature-green fruit in 14 ml of 50 mM CDTA for 6 hr at 23°C (± 1)

[3] Following irradiation fruit were placed in crushed ice until processed

[4] Values within a row followed by the same letter are not significantly different at the 0 05 level by LSD method

of irradiation (Howard and Buescher, 1989, d'Amour et al , 1993) The noted increase in glucose and the decrease in galactose in the present study were consistent with previous reports of significant loss of galactose in irradiated cucumber pickles (Howard and Buescher, 1989) and an increase in glucose in irradiated strawberry fruit (d'Amour et al., 1993) in response to irradiation

Table 4-8 Compositional analysis of neutral sugars[1] in the CDTA-soluble polyuronides[2] derived from pink tomato fruit[3] following irradiation at 0 (control), 0.72 ± 0.11 (low dose), or 1.41 ± 0.15 kGy (high dose)

Treatment	Control	Low dose	High dose
Rhamnose	4.4a[4]	5.1a	4.4a
Arabinose	19.0a	20.8a	21.1a
Xylose	8.0a	9.2a	9.7a
Mannose	3.1a	3.6a	3.5a
Glucose	11.7a	12.8a	12.3a
Galactose	52.8a	48.5a	49.9a
Neutral sugar : uronic acid	0.118a	0.115a	0.116a

[1] Means are the average of three separate derivatizations and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] CDTA-soluble polyuronides were extracted by incubating of 30 mg of AIS derived from irradiated mature-green fruit in 14 ml of 50 mM CDTA for 6 hr at 23°C (± 1)

[3] Following irradiation fruit were placed in crushed ice until processed.

[4] Values within a row followed by the same letter are not significantly different at the 0.05 level by LSD method.

The role of pectin-associated neutral sugars in irradiation-induced softening is still unclear, although the results of the present study show that these sugars remain largely unaffected by irradiation. It is well established that during normal ripening of many fruits the levels of arabinose and galactose associated with pectic polymers (Darvill and McNeil, 1980) decrease (Knee, 1975; Yamaki et al., 1979; Gross and Wallner, 1979). In tomato fruit, 65% of the cell wall galactosyl residues are lost during normal ripening

Table 4-9 Compositional analysis of neutral sugars[1] in the Na_2CO_3-soluble polyuronides[2] derived from mature-green tomato fruit[3] following irradiation at 0 (control),0 72 ± 0 11 (low dose), or 1 41 ± 0 15 kGy (high dose)

Treatment	Control	Low dose	High dose
Rhamnose	5 8a[4]	5 2a	5 3a
Arabinose	19 4a	20 2a	20 5a
Xylose	5 7a	5 9a	6 0a
Mannose	5 4a	5 9a	6 7a
Glucose	7 2a	8 0a,b	9 8b
Galactose	56 5a	54 8a,b	50 9b
Neutral sugar uronic acid	0 116a	0 114a	0 1145a

[1] Means are the average of three separate derivatization , and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] Na_2CO_3 soluble polyuronides were extracted by incubating of 30 mg of AIS derived from irradiated mature-green fruit in 50 mM Na_2CO_3 for 6 hr at 23°C (±1)

[3] Following irradiation fruit were placed in crushed ice until processed

[4] Values within a row followed by the same letter are not significantly different at the 0 05 level by LSD method

(Gross, 1984; Gross and Wallner, 1979 Lackey et al , 1980) Some studies have shown that the loss of neutral sugars during ripening is independent from pectin degradation (Bartley 1976, Gross and Wallner 1979 Knee, 1973). In addition, despite the 50% decrease of galactose reported in fruit of the *rin* mutant, the decrease was not associated with pectin solubilization (Gross and Wallner, 1979), and these mutants soften very slowly (Hobson, 1964) Since the fruit used in these experiments were placed in ice

Table 4-10 Compositional analysis of neutral sugars[1] in the Na_2CO_3-soluble polyuronides[2] derived from pink tomato fruit[3] following irradiation at 0 (control), 0 72 ± 0 11 (low dose) or 1 41 ± 0 15 kGy (high dose)

Treatment	Control	Low dose	High dose
Rhamnose	12.1a[4]	12 9a	12 5a
Arabinose	26 6a	25 9a	26 1a
Xylose	3 9a	4 2a	4 4a
Mannose	2 8a	2 7a	2 6a
Glucose	11 7a	11 8a	11 4a
Galactose	42 9a	42 5a	43 0a
Neutral sugar uronic acid	0 114a	0 1134a	0 1129a

[1] Means are the average of three separate derivatization , and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] Na_2CO_3-soluble polyuronides were extracted by incubating of 30 mg of AIS derived from irradiated pink fruit in 50 mM Na_2CO_3 for 6 hr at 23°C (±1).

[3] Following irradiation fruit were placed in crushed ice until processed

[4] Values within a row followed by the same letter are not significantly different at the 0 05 level by LSD method

immediately following irradiation, quickly processed into EtOH homogenates and exposed to Tris-buffered phenol (Huber 1992) we suggest that the slight effect noted on neutral sugars by irradiation was as a direct impact of irradiation on the structural features of pectins and was not enzymatically mediated Although the trends of neutral sugar change between green and pink tomato fruit control is consistent with earlier reports (Knee 1975, Yamaki et al , 1979, Gross and Wallner, 1979), the role of the pectin-associated neutral sugars in irradiation-induced softening is negligible

Irradiation Effect on Proteins

In order to investigate the effect of irradiation on other features possibly contributing to pectin changes, pericarp tissue including the specific cell wall enzymes and proteins protein from irradiated mature-green and pink tomato fruit were extracted and examined employing SDS-PAGE Proteins extracted from AIS using high salt concentration were examined in mature-green fruit at intervals over a nine-day period following irradiation Total extractable protein was reduced at the high irradiation dose 24 hr following irradiation compared to protein levels in the control and low irradiation-treated fruit (Fig 4-11) However, a significant increase in the protein levels in fruit from both irradiation treatments was observed on the third day compared to the protein levels in the control treatment, and compared to the same treatments on the first day (Fig 4-11) By the fifth day, the protein levels in irradiated mature-green fruit were higher than the protein levels in the control fruit, but decreased to the protein levels of the same treatments observed on the third day (Fig 4-11) Thereafter, there were no significant changes in the levels of extractable protein among the treatments The decrease in protein levels noted on the first day in irradiated mature-green fruit is in agreement with recent reports (Ferullo et al 1994, Triantaphylides et al , 1994) These authors observed an immediate decrease in the capacity of pericarp tissue to incorporate amino acids into proteins following irradiation, suggesting that irradiation adversely affects protein synthesis (Ferullo et al., 1994, Triantaphylides et al 1994) In addition, our results showed an increase in extractable proteins after 24 hours in both irradiation treatments to levels higher than those of the control treatment (Fig 4-11) This is consistent with the

observed recovery in the protein levels from irradiated tomato fruit (Ferullo et al , 1994, Triantaphylides et al. 1994) In contrast to the decrease in extractable protein observed in mature-green fruit within 24 hr following irradiation, levels of extractable proteins from irradiated pink fruit showed an increase within 24 hr following irradiation compared to the non-irradiated pink control treatment (Fig 4-12) The increased levels of extractable protein persisted to the fifth day in irradiated pink fruit, after which time a decrease was noted in fruit from both irradiation treatments compared to the control fruit On the final day of measurement, no significant differences were noted in extractable protein levels among the treatments Most of the data related to irradiation of proteins in aqueous solutions support the view that the produced free radicals in water are responsible for the change in the shape of the protein molecule (unfolding) facilitating the aggregation process and the subsequent increase in the molecular weight However, the physical and chemical changes in bovine serum albumin (contained 5% water) induced by irradiation reported by Alexander and Hamilton (1960) indicated the increased in the amount of the protein with no alteration to the molecular weight.

To further investigate the effect of irradiation on tomato fruit proteins and to test for changes in PG2 (isoenzyme 2) levels associated with the fluctuation in the extractable protein levels, SDS-PAGE analysis was conducted

No bands corresponding to PG2 protein (MW 44-45 kD) were apparent on the first day in all treatments of mature green fruit, in agreement with our results indicating that PG activity was not detected in mature-green fruit following irradiation (Chapter 3 - Fig 3-7), and with other reports that PG enzyme is absent at this stage of development

(Hobson, 1964; Buescher and Tigchelaar, 1975) On the fifth day, bands representing PG2, confirmed via western blot analysis (Chun and Huber unpublished data) were observed in both control and irradiated fruit, although with lesser intensity in the higher irradiation treatment (Fig 4-13, A) At the tenth day, differences between the control and both irradiation treatments were noted, with the control sample yielding a more intensive PG band, which confirms our findings reported earlier in Chapter 3 (Fig 3-7) indicating the reduction in PG activity in irradiated fruit compared to the control These results indicate that irradiation might act by suppressing the accumulation of PG enzyme at an early stage of fruit development.

SDS PAGE of salt-extractable protein from pink tomato fruit showed the presence of PG2 in fruit of all treatments at all times measured (Fig 4-13, B) That no major differences in PG protein were noted for pink fruit is in agreement with the findings reported in Chapter 3 (Fig 3-8) regarding the trends of PG activity in pink fruit The intensity of these bands were almost the same, however, the reduction in PG enzyme activity noted in chapter 3 (Fig 3-8), indicates that irradiation may reduce PG activity via enzyme inactivation in addition to adversely affecting the protein accumulation It has been postulated that irradiation of tomato fruit at 0 5-3 kGy stimulated the synthesis of the so called gamma-induced proteins (GIPs) (Ferullo et al , 1994, Triantaphyides et al , 1994)

This work indicates clearly that irradiation selectively affected some proteins by decreasing and/ or increasing their synthesis (judged by their decrease or increase in activity and/ or presence, absence and intensity of bands in SDS-PAGE), and that this

effect was either direct, influencing protein structure or indirect via free radicals produced by irradiation

Conclusions

The effect of irradiation on the cell wall matrix polymers was via direct and/ or indirect mechanism(s) that triggered the breakdown of cell wall structure, resulting in the noted tissue softening of tomato fruit The decrease in the total uronic acid content in the higher irradiation dose treatments, the increased solubility and the downshifting of the M_r of the H_2O- and CDTA-soluble polyuronides, associated with only slight influences on the neutral sugars and hemicellulose, indicate that the pectic fractions were the most affected by irradiation Therefore, we assume that the degradation of the pectic fractions was the major event characterizing irradiation-induced tissue softening in tomato fruit The changes noted in the proteins, on the other hand, might be partially responsible for the disturbed pattern of the normal ripening, resulting in irregular ripening in addition to other effects on their contribution to enhance and/ or regulate tissue softening The effect of irradiation was more severe in mature-green than in pink fruit, indicating that the effect of irradiation on the younger tissue might have triggered the syntheses of the proteins and enzymes that regulate the normal development of the fruit, in addition to the drastic effect on the cell wall structure.

There was no apparent differences between pericarp and locular gel tissue in terms of their response to irradiation. Both types of tissue exhibited an increase in polyuronide solubility and downshifting of the M_r. However, since pericarp tissue

proportionally contains more cell wall materials than locular gel tissue it is considered to have more impact on the softening of the fruit

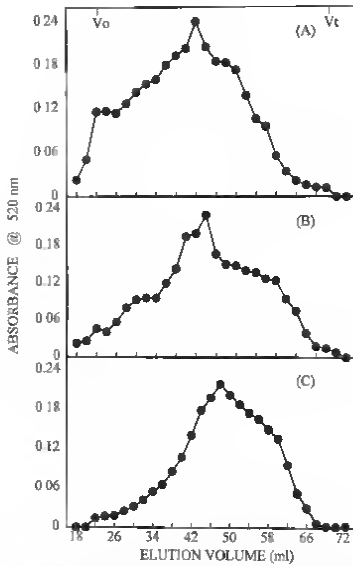


Figure 4-1 Sepharose CL-2B-300 profiles of H_2O-soluble polyuronides derived from mature-green tomato fruit following irradiation at 0 (A), 0 73 ± 0 11 (B), or 2 21 ± 0 22 kGy (C) Vertical tick marks at the top of profile A indicate the elution positions of standards blue dextran 2,000 kDa (void volume, V_o) and glucose (total volume, V_t) Absorbance at 520 am colorimetric determination of galcturonic acid equivalents.

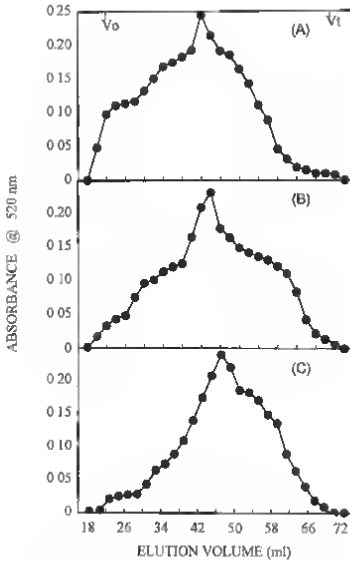


Figure 4-2 Sepharose CL-2B-300 profiles of H_2O-soluble polyuronides derived from mature-green tomato fruit irradiated at 0 (A), 0 72 ± 0 11 (B) or 1 41 ± 0 15 kGy (C) Vertical ticks (Vo & Vt) are as for Figure 4-1

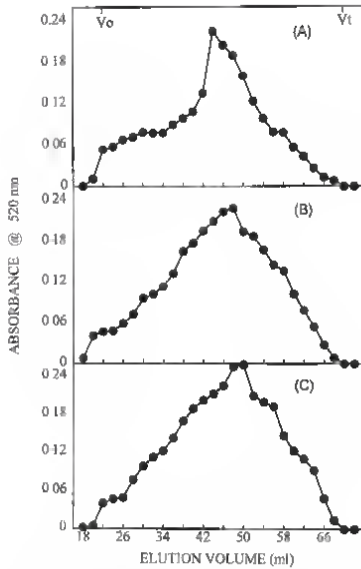


Figure 4-3 Sepharose CL-2B-300 profiles of H_2O-soluble polyuronides derived from pink tomato fruit irradiated at 0 (A) 0 73 ± 0 11 (B) or 2 21 ± 0.22 kGy (C) Vertical ticks (Vo & Vt) are as for Figure 4-1.

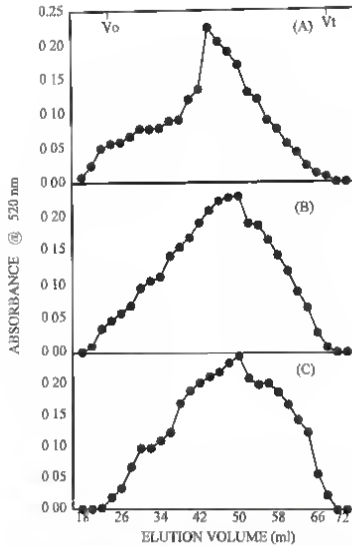


Figure 4-4 Sepharose CL-2B-300 profiles of H2O-soluble polyuronides derived from pink tomato fruit following irradiation at 0 (A), 0 72 ± 0 11 (B) or 1 41 ± 0 15 kGy (C) Vertical ticks (Vo & Vt) are as for Figure 4-1

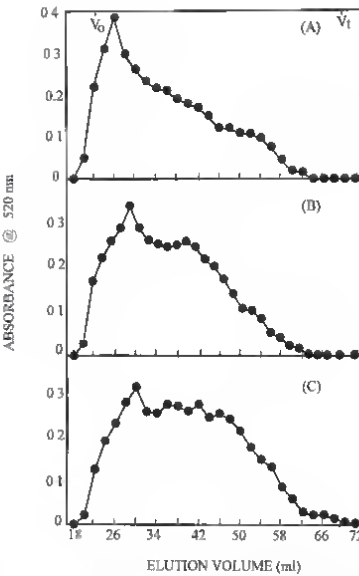


Figure 4-5 Sepharose CL-2B 300 profiles of CDTA soluble polyuronides derived from mature green tomato fruit following irradiation at 0 (A), 0 73 ± 0 11 (B) or 2.2 t ± 0.22 kGy (C) Vertical ticks (Vo & Vt) are as for Figure 4-1

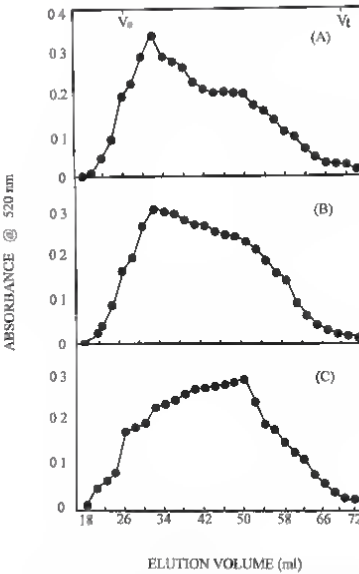


Figure 4-6 Sepharose CL-2B-300 profiles of CDTA-soluble polyuronides derived from pink tomato fruit following irradiation at 0 (A), 0 73 ± 0 11 (B) or 2 21 ± 0.22 kGy (C) Vertical ticks (Vo & Vt) are as for Figure 4-1

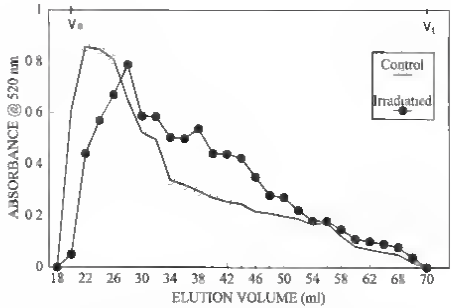


Figure 4-7 Sepharose CL-2B-300 profiles of CDTA-soluble polyuronides derived from pericarp tissue of PG antisense tomato fruit irradiated at 0 (control) or 2 675 ± 0 075 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 4-1

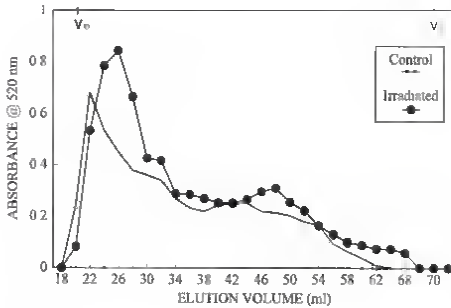


Figure 4-8 Sepharose CL-2B-300 profiles of CDTA-soluble polyuronides derived from AIS from locular gel tissue of antisense tomato fruit irradiated at 0 (control) or 2 675 ± 0 075 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 4-1

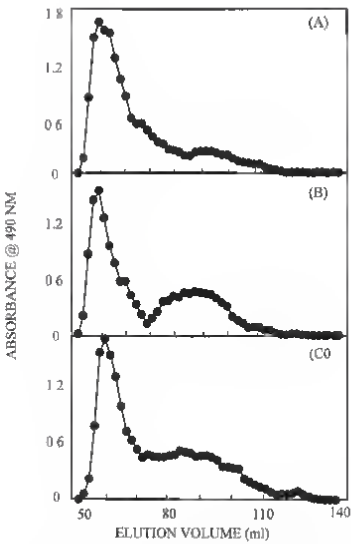


Figure 4-9· Sepharose CL-2B-200 profiles of hemicellulose derived from mature-green tomato fruit followng irradiation at 0 (A), 0 72 ± 0 11 (B) or 2 21 ± 0 22 (C) kGy

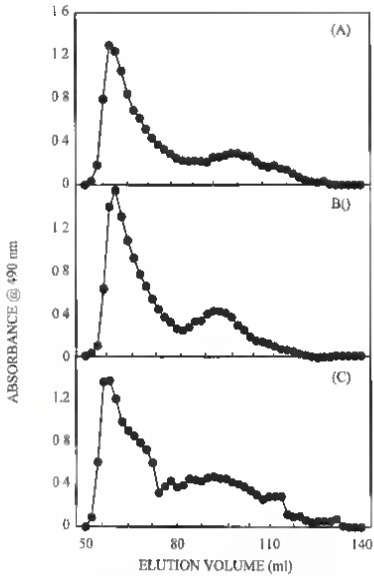


Figure 4-10 Sepharose CL-4B-200 profiles of hemicellulose derived from pink tomato fruit following irradiation at 0 (A), 0 73 ± 0 11 (B) or 2.21 ± 0.22 kGy (C)

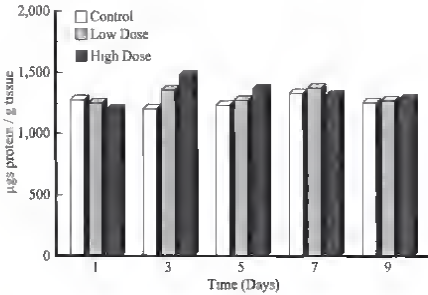


Figure 4-11 Total salt-extractable proteins in the AIS derived from pericarp tissue of mature-green tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose) or 1 41 ± 0 15 kGy (high dose) Protein samples were prepared every other day over a nine-day storage period at 20°C Units are expressed as µg total extractable protein per gram tissue fresh weight. LSD = 36 165

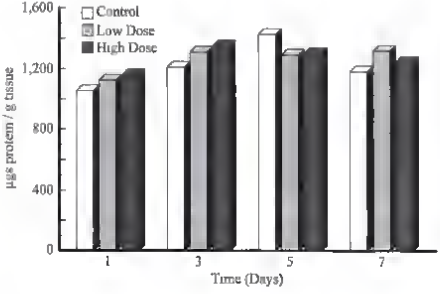


Figure 4-12 Total salt-extractable proteins in the AIS derived from pericap tissue of pink tomato fruit following irradiation at 0 (control), 0 72 ± 0 11 (low dose) or 1 41 ± 0.22 kGy (high dose) Protein samples were prepared every other day over a seven-day storage period at 20°C Units are expressed as µg total extractable protein per gram tissue fresh weight LSD = 53 65

Fig 4-13 SDS-PAGE of total cell wall proteins from 'Sunny' tomato fruit pericarp extracted 1, 5 and 10 days from mature-green (A), and 1, 5 and 7 days from pink fruit (B) following irradiation and storage at 20°C

(A)

(B)

CHAPTER 5
IRRADIATION-INDUCED DEGRADATION OF TOMATO CELL WALL COMPONENTS IN VITRO- IMPLICATIONS FOR THE MECHANISM OF FRUIT SOFTENING

Introduction

In vitro systems have been the traditional approach employed in studying cell wall modifications that occur during normal ripening of fruit. Cell wall preparations have been used extensively to study enzyme activity and the interactions of enzymes with cell wall polymers. *In vitro* systems might provide some worthwhile information or general guidelines about the nature of the changes or responses that occur *in vivo* (in response to a certain provocation) and might lead to better understanding of the subject under investigation. However, *in vitro* systems might, in some cases, give a very severe response to a certain event that does not represent the actual response that occurs *in vivo*. For example, evidence indicates that polyuronide degradation *in vivo* is much less extensive than that observed *in vitro* (Seymour et al., 1987).

In vitro systems have long been employed in studying the effects of irradiation on cell wall polymers. Dwight and Kersten (1938) were the first to introduce such a system when they exposed "pure" apple pectin to X-rays, and found that the pectin was subject to extensive degradation as indicated by lower solution viscosity.

No reports were found in the literature dealing in particular with direct irradiation of protein extracts or PG2 enzyme from tomato fruit. Data obtained in the previous experiments (Chapters 3 and 4) demonstrated that irradiation of mature-green and ripening tomato fruit resulted in increased electrolyte efflux, pectin solubilization, and pectin depolymerization. These events may explain the effect of irradiation in inducing both short- and long-term decreases in firmness of both whole fruit and excised pericarp discs (Chapter 3), but did not provide direct evidence for the mechanism of the textural changes. Irradiation-induced pectin solubilization and depolymerization may occur via a number of mechanisms, both direct and indirect. The former would include the capacity of irradiation to directly damage one or more linkages of pectin polymers. Effects on pectin structure might also occur indirectly, such as by way of active radicals produced via irradiation-induced damage to cellular membranes, or via the action of specific enzymes whose activity or synthesis might respond positively to irradiation.

The main objective of this study was to examine whether the irradiation-induced effects on pectin occur via direct or indirect mechanisms or both types. To address this issue, tomato AIS, and commercial samples of pectin, polygalacturonic acid, and cellulose were individually subjected to direct irradiation and examined for changes in molecular mass and solubility. The carbohydrate samples were also supplemented with oleic and linoleic acids to determine whether products of radiolytic cleavage were involved in irradiation-induced pectin changes. To test whether tissue enzymes were involved in the irradiation effects on pectic polymers, comparative studies of the effects of irradiation were performed on AIS isolated in enzymically active form and AIS treated

to eliminate associated hydrolase activity (phenol treated). Finally, the study also examined the effects of irradiation on cell-free protein extracts of mature-green and pink tomato fruit, and on crude and pure polygalacturonase and β-galactosidase.

Materials and Methods

Plant Material

Tomato fruit (*Lycopersicon esculentum* L. cv Sunny) at mature green and pink stages of development were harvested as described in Chapter 3. However, in the experiments described herein, AIS derived from these fruit rather than the intact fruit were exposed to irradiation.

Alcohol-insoluble solids preparations. Alcohol-insoluble solids (AIS) from the mature-green and pink tomato fruit were prepared as described by Huber (1991) with slight modifications (Huber and O'Donghue, 1993) as described in Chapter 4. One group AIS homogenate was washed thoroughly with cold 80% EtOH (non-BP-treated); the second was treated with Tris-buffered phenol (BP-treated) to inactivate tissue hydrolases (Huber, 1992). Both groups were oven dried at 37°C overnight.

1- Four samples (100 mg each) of the non BP-treated AIS were moistened with 1-2 ml 80% EtOH each to prevent clotting, suspended in 20 ml of dt H_2O, and exposed to irradiation as described below.

2- Twelve samples (100 mg each) of AIS BP-treated to inactivate enzymes, were moistened with 1-2 ml 80% EtOH each, and suspended in 20 ml dt H_2O. Twenty mg of either linoleic (18:2, Δ 9,12) or oleic (18:1, Δ 9) fatty acids (Sigma) were added to eight

of the BP-treated samples and the samples exposed to irradiation. Following irradiation, the AIS suspensions were filtered with Miracloth, the filtrates centrifuged at 15,000 × g and filtered again with Miracloth. The supernatants were then analyzed for soluble polyuronides as described below.

3- In a separate experiment, eight samples of non-BP-treated and BP-treated AIS were prepared as above, but without the addition of fatty acids and subjected to irradiation.

4- Eight dry samples of BP- and non-BP treated AIS preparations were exposed to irradiation, without moistening or suspension in di H_2O. Following irradiation, the samples from the third and fourth experiments were filtered, centrifuged, the supernatants analyzed for soluble polyuronides, and the pellets incubated in CDTA for 6 hr at 23°C (±1), then analyzed as described below.

Commercial samples of pectin, polygalacturonic acid, and cellulose. Six samples of 50 mg each of citrus polygalacturonic acid, pectin (polygalacturonic acid methyl ester) (Sigma), and Avicel cellulose were suspended in 20 ml di H_2O and subjected to gamma irradiation.

Salt-extractable protein preparations. Protein extraction was conducted as described in Chapter 4. Two protein samples from non-irradiated fruit at the mature-green and pink stages of development, each in 100 ml Trizma base buffer were exposed to irradiation as described below.

Purified polygalacturonase2 enzyme. Polygalacturonase2 (PG2) enzyme preparation extracted according to Huber and O'Donoghue(1993) was provided by Dr. D. Huber's lab at the University of Florida, Gainesville, FL. One-half ml of buffer

containing 240 µg PG2 was diluted to 6 ml with di H_2O and subdivided into two, 3-ml samples One sample was then exposed to irradiation as described below

Irradiation Treatments

For all experiments irradiation was performed at the Florida Department of Agriculture and Consumer Services Division of Plant Industry (DPI) facilities in Gainesville, Fl using gamma rays emitted from a cesium source (Gammacell-100, AECL, Canada) Screw top test tubes (50 ml) containing AIS protein, or commercial polymer sample were placed in a cardboard cylinder designed to fit in a rotating irradiation chamber and subjected to irradiation at doses of 0 or 1 0 ± 0 04 kGy Following irradiation, all samples were immediately placed in crushed ice until processed

Polyuronide Analysis

Uronic acid analysis. Total uronic acid content in the AIS was determined as described by Ahmed and Labavitch (1977) Preparation of specific pectic fractions was as described in Chapter 4

Total polysaccharide determination Total polysaccharide content was measured using the phenol-sulfuric method (Dubois et al , 1956) Briefly, 0 5 ml phenol were added to a 0 5 ml aliquot of the sample, followed by addition of 2.5 ml of concentrated sulfuric acid. After cooling, the samples were measured spectrophotometrically at 490 nm

Gel chromatography. Molecular size analysis of H_2O- and CDTA-soluble pectic fractions was carried out as described in Chapter 4

Compositional Analysis of Pectin Associated Neutral Sugars

Neutral sugar compositional analysis was carried out as described by Blakeney et al. (1983), with the modifications described in Chapter 4

Protein and Enzyme Activity Assays

Total salt-extractable proteins and enzyme activity determinations were carried out as described in Chapters 3 and 4.

Statistical Analysis

Data from the three irradiation treatments were analyzed as a completely randomized design with three replicates Data obtained from each fruit maturity stage were analyzed separately using Analysis of Variance (ANOVA), and means were separated by the Least Significant Difference method at the 0 05 level

Results and Discussion

Influence of Irradiation on the Pectic Components of Enzymically Active and Inactive Alcohol-Insoluble Solids

Non-BP- and BP-treated AIS. In view of the decrease in total uronic acid content and increase in polyuronide solubility in irradiated fruit relative to the controls reported in Chapter 4 (Tables 4-1, 2, and 3), experiments were conducted to study the effect of irradiation on cell wall pectic fractions. Emphasis was placed on two mechanisms direct (targeting cell wall polymers directly) and indirect (via enzymic reactions and/ or generation of radiolytic products from other cellular components, including membranes).

An increase of 70% in H_2O-soluble polyuronides from irradiated non-BP-treated (enzymically active) samples derived from mature-green fruit was noted compared to the control (Table 5-1) Irradiated BP-treated (enzymically inactive) samples and BP treated samples provided with oleic acid (BP treated + O) or with linoleic acid (BP treated + L) exhibited 92% 80%, and 70% increases in soluble polyuronides, respectively, compared to the non-irradiated controls (Table 5-1) There were no significant differences among the irradiated BP-treated samples, indicating that irradiation-induced membrane damage, while clearly a response to irradiation treatment (Chapter 3, Tables 3-1 and 2), was not responsible for the effects of irradiation on polyuronides. The proportionally lower total uronic acid content of AIS not exposed to phenolic solvents is likely due to the higher proportion of non-cell wall components in the non BP-treated samples from mature-green A 25% increase in soluble polyuronides was noted in the irradiated non-BP-treated AIS from pink fruit compared to the control (Table 5-1) Similarly, increases of 24%, 25% and 32% in soluble polyuronides were noted in the BP-treated, BP-treated+ O and BP-treated + L samples, respectively, compared to the controls (Table 5-1) A notable observation was the dramatically higher levels of polyuronides in the non-BP-treated samples from pink fruit compared to the BP-treated samples, either in the non irradiated control sample (0 kGy) or in the irradiated (1 0 ± 0 04 kGy) sample Since the non-BP-treated samples contain most of the cell wall materials, including cell wall bound proteins (Huber 1992) and were suspended in water prior to and during irradiation for a duration of 2-3 hr at 23°C (±1) (sufficient time and optimum temperature

Table 5-1 H_2O-soluble polyuronides[1] derived from AIS homogenates of pericarp tissue of mature-green and pink tomato fruit either enzymically active (non-BP-treated), or enzymically inactive (BP-treated), with or without addition of fatty acids and irradiated with gamma rays at 0 or 1 0 ± 0 04 kGy

AIS preparation		H_2O-soluble polyuronides	
		Non-irradiated	Irradiated
Mature green			
	Non-BP-treated	22.9c[2]	39 7b
	BP-treated	22.7e	43 6a
	BP-treated + O[3]	24 6c	44 2a
	BP-treated + L[4]	25 0c	43 0a
Pink			
	Non-BP-treated	91 0b	114a
	BP-treated	44 0d	52 0c
	BP-treated + O	43 0d	54 0c
	BP-treated + L	40 0d	53 0c

[1] Expressed as µg uronic acid/mg AIS

[2] Values followed by the same letter within each maturity stage are not significantly different at the 0 05 level by LSD method

[3] BP treated+ O Buffered-phenol treated AIS provided with oleic acid (8 1, ▵ 9)

[4] BP-treated+ L Buffered-phenol treated AIS provided with linoleic acid (18.2, ▵ 9,12)

for enzyme reactions to occur), it is most likely that enzymic reactions contributed to the increased levels of soluble polyuronides noted in these samples. In addition, the difference noted between the non-BP-treated and BP treated samples provides conclusive

evidence that the increased solubility in the irradiated BP-treated samples was solely a result of irradiation and no enzymatic involvement is likely to have occurred

To confirm the elimination of any enzymatic involvement and to test further the direct impact of irradiation on solubility properties of polyuronides, another experiment was conducted using both types of preparations as above. After filtration, the supernatant was analyzed for H_2O-soluble polyuronides, and the pellet was incubated in CDTA for 6 hr at 23°C (±1), filtered, and the CDTA-soluble polyuronides analyzed. Lower levels of total uronic acids were noted in non-BP-treated samples from both mature-green and pink AIS compared to BP treated samples (Table 5-2). Non-irradiated non-BP-treated samples were 7% and 21% lower in total uronic acid content than BP-treated samples from mature-green and pink fruit, respectively (Table 5-2). Total uronic acid content was determined in the samples prior to irradiation, because irradiation was performed while the samples were suspended in H_2O, which precluded determination of uronic acid content. The higher total uronic acid content in mature-green BP-treated AIS samples could be attributed to the absence of non-cell wall components, which make it proportionally more pure compared to non-BP-treated samples. While this is also true for the AIS samples from (ripening) pink fruit, the greater difference noted here between non-BP-treated and BP-treated samples implies that some enzymatic reactions occurred in the non-BP-treated samples. On the other hand, this provides additional evidence of the absence of enzyme involvement in BP treated samples, confirming that the effect noted on total uronic acid content is a consequence of irradiation

Table 5.2 Total uronic acid content, H_2O-, and CDTA-soluble polyuronides[1] derived from AIS homogenates of pericarp tissue of mature-green and pink tomato fruit either enzymically active (non-BP-treated) or enzymically inactive (BP-treated) and irradiated with gamma rays at 0 or 1.0 ± 0.04 kGy

AIS preparation	Total uronic acid	Soluble polyuronides	
		H_2O-	CDTA
Mature green			
Non-BP-treated	294b[2,3]		
Non-irradiated	N/D[4]	22.8c	47.0b
Irradiated	N/D	37.3b	67.5a
BP-treated	314a		
Non-irradiated	N/D	25.6c	47.0b
Irradiated	N/D	45.0a	62.0a
Pink			
Non-BP-treated	259b		
Non-irradiated	N/D	83.0b	61.0a
Irradiated	N/D	102.0a	60.0a
BP-treated	328a		
Non-irradiated	N/D	42.0d	48.5b
Irradiated	N/D	52.4c	53.5a,b

[1] Expressed as µg uronic acid/mg AIS.

[2] Values followed by the same letter within each maturity stage are not significantly different at the 0.05 level.

[3] Total uronic acid was determined for the AIS preparations prior to irradiation.

[4] Total uronic acid was not determined after suspension of the sample in H_2O.

The tendency of the soluble polyuronide content in the supernatant (H_2O-soluble) to be greater in irradiated samples from mature-green fruit (Table 5-2) was similar to that noted above (Table 5 1) Similarly, CDTA-soluble polyuronides were higher by 44% and 32% in the irradiated, non-BP-treated and BP-treated samples, respectively, derived from AIS of mature-green fruit, with no significant differences between non-BP-treated and BP-treated samples (Table 5.2) There were no significant differences in the CDTA-soluble polyuronides noted between non-irradiated control samples and irradiated samples of non-BP-treated preparations form pink fruit (Table 5-2), probably indicating thet most of the soluble polyuronides that might have resulted from the direct impact of irradiation were already included in the dramatically increased H_2O-soluble fraction A slightly significant difference was noted in the CDTA-soluble polyuronides in the BP-treated preparations from control and irradiated samples from pink fruit (Table 5-2) There were no significant differences between levels of CDTA-soluble polyuronides in irradiated non BP-treated samples from pink fruit (Table 5-2)

Irradiation had no effect on the total uronic acid content of irradiated, non-BP-treated or BP-treated samples from mature-green fruit (Table 5 3) However the total uronic acid levels in the non-BP-treated samples were lower than those of the BP-treated samples

To test whether the degradation of pectin occurred directly or indirectly (via free radicals), water, which would presumably facilitate the formation of free radicals, was eliminated and dry pectin was directly irradiated No significant changes were noted in

Table 5-3 Total uronic acid content, H_2O-, and CDTA-soluble polyuronides[1] derived from AIS homogenates of pericarp tissue of mature-green and pink tomato fruit either enzymically active (non-BP-treated), or enzymically inactive (BP-treated) allowed to dry, and irradiated with gamma rays at 0 or 1 0 ± 0 04 kGy

AIS preparation	Total uronic acid	Soluble polyuronides	
		H_2O-	CDTA-
Mature green			
Non-BP-treated			
Non-irradiated	293a[2]	27 0c	58 0a
Irradiated	291a	42 0a,b	60 0a
BP-treated			
Non-irradiated	328b	36 0b	47 0b
Irradiated	334b	45 0a	61 0a
Pink			
Non-BP-treated			
Non-irradiated	304b	96 0a	74 0a
Irradiated	271c	100a	75 0a
BP-treated			
Non irradiated	384a	54 0b	53 0b
Irradiated	369a	57 0b	60 0b

[1] Expressed as µg uronic acid/mg AIS

[2] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0 05 level by LSD method

total uronic acid from irradiated mature-green, non-BP-treated and BP-treated samples compared to the controls (Table 5-3) However, significant differences in total uronic acid content existed between non-BP-treated samples (irradiated and non-irradiated) and BP-treated samples (irradiated and non irradiated), with the latter being higher (Table 5-3)

Total uronic acids in irradiated non BP-treated dry samples from AIS of pink fruit were higher than the control (Table 5-3) This might be attributed to the possibility that irradiation affected the proteins (during irradiation) in the non-BP-treated samples, preventing further loss of total uronic acid, at which time the control samples were incubated at 23°C (±1) during the irradiation period.

An increase in solubility was noted in the H_2O-soluble polyuronides in irradiated dry samples of both non-BP treated and BP treated preparations from AIS of mature-green fruit compared to the controls (Table 5-3) No significant differences were noted in all other treatments of dry samples

Results indicate that the degradation of dry, non-BP-treated and BP-treated samples by irradiation was less pronounced than when the samples were irradiated in solution This is in agreement with an early report of irradiated commercial citrus pectin (Kertesz et al , 1956), although our results show that the effect was not as drastic as was reported previously The increase in solubility in the H_2O-soluble polyuronides of irradiated dry samples of mature-green and pink non-BP-treated AIS, and the lower amount of total uronic acid in the irradiated pink non-BP-treated AIS compared to the non-irradiated indicate that the effect of irradiation in the absence of water occurred

directly and not as a secondary effect via free radicals Therefore, this suggests that the direct effect of irradiation is also likely to occur, at least partially, in the *in vivo* system

Irradiation Effects on Commercial Preparations

As an additional assessment of the specificity of irradiation effects on structural polysaccharides, polygalacturonic acid, pectin (esterified), and cellulose were individually irradiated at 0 or 1 0 ± 0 04 kGy A preliminary phenol-sulfuric acid test indicated that Avicel, a crystalline form of cellulose, exhibited no apparent response to irradiation at 1 0 ± 0 04 kGy Irradiated pectins showed higher absorbance values than control (control = 1.391 vs irradiated =1.521) by Blumenkrantz and Asboe-Hansen (1973) method, an indication that irradiated samples contain more polysaccharides than the non-irradiated with less intense color Whereas, the absorbance of irradiated polygalcturonic acid (0.968) was slightly higher than the control (0 873), indicating that there was no effect of irradiation on the polysaccharides These absorbance values indicate that the pectin was altered by irradiation, and further testing was conducted using gel filtration chromatography to determine any changes in the M, of both pectin and polygalcturonic acid samples

Molecular Weight Analysis of Soluble Polyuronides

Sepharose-CL-2B 300 profiles of H2O-soluble fractions derived from non-BP-treated AIS samples mature-green fruit irradiated at 0 or 1 0 ± 0 04 kGy clearly shown a downshifting of M, at 1 0 ± 0 04 kGy compared to the control (Fig 5-1) This downshifting of the irradiated, non-BP-treated samples was not as pronounced as that noted in the irradiated, BP treated sample, which exhibited a dramatic M, downshifting in

response to irradiation compared to the control (Fig 5-2) This difference in the magnitude of the downshifting between the two samples may be related to the differences in the purity of the preparations In the non-BP-treated sample, that contains non-cell wall materials the distribution of the M, size of the pectic fragments in the control sample is broader relative to the control BP-treated sample (Fig. 5-1) On the other hand, the downshifting of the irradiated sample of BP-treated was more dramatic compared to its control and to the non-BP treated samples due to the purity of the sample (i e. contains more pectic substances proportional to its weight).

The same tendency of the downshifting in M, was also noted in irradiated non-BP-treated and BP-treated samples from pink fruit compared to the control (Figs 5-3 and 5-4) In addition, differences also existed between non-BP-treated and BP treated samples from pink fruit in the magnitude of the downshifting noted. It is believed that the more pronounced downshifting noticed in the non-BP-treated samples was in response to enzymatic reactions that did not occur in BP treated samples Where as the lower M, noted in all pink AIS samples compared to the mature-green samples is due to the inherently lower M, of the soluble polyuronides that is characteristic in pink fruit compared to mature-green fruit

Sepharose CL-2B-300 profiles of the CDTA-soluble fractions derived from non-BP-treated and BP-treated samples from mature-green fruit irradiated at 1 0 ± 0 04 kGy showed a considerable downshifting in M, relative to the controls (Figs 5-5 and 5-6) A similar tendency was also noted in profiles of CDTA-soluble pectic fractions from pink fruit AIS, non-BP-treated and BP-treated irradiated at 1 0 ± 0 04 kGy relative to the

controls (Fig. 5-7 and 5-8). The differences in the M_r distribution and the magnitude of downshifting in CDTA-soluble pectic fractions between non-BP-treated and BP-treated samples were not as pronounced as those observed earlier in the profiles of H_2O-soluble pectic fractions.

Sepharose-CL-2B-300 profiles of H_2O-soluble pectin fractions derived from commercial (esterified) pectin irradiated at 1.0 ± 0.04 kGy showed downshifting in the M_r relative to the control (Fig. 5-9), although both profiles showed considerable downshifting compared to all the samples from tomato discussed so far. In contrast, Sepharose-CL-2B-300 profiles of H_2O-soluble fractions derived from polygalacturonic acid (deesterified) irradiated at 1.0 ± 0.04 kGy showed no changes in their M_r in response to irradiation and were almost identical to the control (Fig. 5-10). The observation that deesterified pectin was less affected by irradiation compared to the esterified pectin might indicate that esterified pectins are more susceptible to irradiation.

Compositional Analysis of Pectin-Associated Neutral Sugars

The direct *in vitro* exposure of pectin samples to irradiation had a dramatic effect on neutral sugar composition that was far more extensive than the effect on neutral sugars in irradiated intact fruit. Neutral sugar analysis revealed 4.5% and 13% reductions in total neutral sugars of H_2O-soluble polyuronides from mature-green AIS, non-BP-treated and BP-treated, samples irradiated at 1.0 ± 0.04 kGy, respectively, compared to the control (Table 5-4). Considerable losses of arabinose and galactose, and an increase in xylose and glucose were noted both irradiated preparations compared to their

Table 5-4 Neutral sugar analysis of H_2O-soluble polyuronides from non-phenol treated (non BP-treated) or Tris buffered phenol treated (BP treated) mature-green homogenates irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated)

Neutral sugars	Non BP-treated		BP-treated	
	Non-irrad	Irrad.	Non-irrad	Irrad.
Rhamnose	2 89a	2 19a	2 34a	2 67a
Arabinose	16 2a,b	11 9b	18 7a	12 4b
Xylose	1 1c	3 3b	4 4b	7 7a
Mannose	4 8b	4 2b	5 2a,b	6 4a
Glucose	29 7a	35 8a	19 5b	29 6a
Galactose	46 3a	41 1b	48 7a	40 0b
Neutral sugar : uronic acid[2]	0 181a	0 173b	0 13c	0 113d

Values are the average of three separate derivatizations , and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] Means in a row followed by the same letter are not significantly different at the 0 05 level by LSD method.

[3] Expressed as µmol ratio

non-irradiated controls (Table 5-4), but the changes in arabinose and glucose were statistically significant only in the BP-treated samples

Total neutral sugars decreases of 19% and 7% were noted in H_2O-soluble polyuronides non-BP-treated and BP-treated AIS samples, respectively, in pink fruit compared to the non-irradiated controls (Table 5-5) While there were losses in arabinose and galactose in samples from both non-BP-treated and BP treated AIS, and increases in

Table 5 5 Neutral sugar analysis of H2O-soluble polyuronides from non-phenol treated (non-BP-treated) or Tris buffered phenol treated (BP-treated) pink homogenates irradiated at 0 (control), or 1 0 ± 0 04 kGy (irradiated)

Nautral sugars	Non BP-treated		BP-treated	
	Non-irrad	Irrad.	Non-irrad.	Irrad.
Rhamnose	2 8b	2 9b	5 3a	6 0a
Arabinose	16 0c	12 5d	27 64a	22 8b
Xylose	6 7b	8.3b	8 3b	11 5a
Mannose	6 0b	9 4a	9 6a	7 0a,b
Glucose	20 0c	35 1a	24 4c	30 8b
Galactose	30 0b	27 8b	41 2a	27 8b
Neutral sugar : uronic acid	0 167b	0 135c	0 182a	0 169a

[1] Means are the average of three separate derivatization , and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] Values in a row followed by the same letter are not significantly different at the 0 05 level by LSD method

[3] Expressed as µmol ratio

mannose and glucose in H_2O-soluble polyuronides of non-BP-treated AIS samples from pink fruit compared to the control (Table 5-5), the xylose and glucose levels increased in BP-treated samples.

Total neutral sugars of CDTA-saluble polyuronides from mature-green AIS preparations of non BP treated and BP-treated irradiated samples exhibited 15% and 19% reductions, respectively, compared to non-irradiated controls (Table 5-6) Losses of arabinose, galactose, and an increases in mannose and glucose occurred in both irradiated

Table 5-6 Neutral sugar analysis of CDTA-soluble polyuronides from either no-BP-treated (non-BP-treated) or Tris buffered phenol treated (BP-treated) mature-green homogenates irradiated at 0 (control), or 1 0 ± 0 04 kGy (irradiated)

Neutral sugars	Non-BP-treated		BP-treated	
	Non-irrad	Irrad.	Non-irrad	Irrad.
Rhamnose	4 6a,b	5 6a	4 3a,b	3 4b
Arabinose	13 1c	10 5d	25 6a	19 0b
Xylose	14 2a	15 5a	17 5a	18 3a
Mannose	9 5a	7 6b	5 0c	6 5b
Glucose	27 3a,b	37 1a	25 0b	33 3a,b
Galactose	30 1a,b	26 6b	32 5a	19 5c
Neutral sugar uronic acid[2]	0 189a	0 161b	0 170a,b	0 150c

[1] Means are the average of three separate derivatizations , and are expressed as mole % of individual neutral sugar to total neutral sugars

[2] Values in a row followed by the same letter are not significantly different at the 0 05 level by LSD method

[3] Expressed as µmol ratio

preparations compared to non-irradiated controls (Table 5-6) Reductions of 19% and 7% in total neutral sugars were observed in CDTA-soluble polyuronides from pink fruit AIS of non-BP-treated and BP-treated irradiated samples, respectively, compared to the non-irradiated controls (Table 5-7), but the only significant change among the individual neutral sugars was a loss of galactose in the BP-treated samples

Table 5-7 Neutral sugar analysis of CDTA-soluble polyuronides from non-phenol treated (non-BP-treated) or Tris buffered phenol treated (BP-treated) from pink homogenates irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated)

Neutral sugars	Non-BP-treated		BP-treated	
	Non-irrad	Irrad.	Non irrad	Irrad
Rhamnose	3 8a,b	4 3a	3 5b	3 3b
Arabinose	20 4a,b	15 5b	22 6a	23 5a
Xylose	8 2b	9 4a,b	10 0a,b	11 8a
Mannose	7 9a	6 8a	6 0a	7 2a
Glucose	26 9b	34 8a,b	29 0a,b	35 8a
Galactose	32 1a	25 9a,b	29 3a	19 7b
Neutral sugar : uronic acid[3]	0 182a	0 148b	0 124c	0 115d

[1] Means are the average of three separate derivatizations and are expressed as mole % of individual neutral sugar to total neutral sugars.
[2] Values in a row followed by the same letter are not significantly different at the 0 05 level by LSD method.
[3] Expressed as µmol ratio

Irradiation Effects on Proteins and Enzymes

In order to study the direct impact of irradiation on proteins, which might give some indications to explain the dramatic effect of irradiation on the extractable proteins from irradiated fruit reported earlier in Chapters 3 and 4, salt-extractable protein from mature-green and pink AIS preparations were directly irradiated at 0 or 1 0 ± 0 04 kGy Considerable increases in total extractable proteins were noted in both irradiated protein samples relative to the non-irradiated control samples (Table 5-8) The direct impact of

Table 5-8 Total amount of proteins and total enzyme activity of irradiated protein extract derived from mature green and pink tomato fruit.

Treatment	Protein[1]	Total activity[2]	
		Polygalacturonase	β-galactosidase
Mature green			
Non-irradiated	1675b[3]	ND	0 0350a
Irradiated	1925a	ND	0 0353a
Pink			
Non-irradiated	1605b	0 618a	0 0318a
Irradiated	2115a	0 307b	0 0322a
Pure PG enzyme[4]			
Non-irradiated		4 85a	
Irradiated		3 35b	

[1] Expressed as μg^{-1} g $^{-1}$fresh weight
[2] Expressed as μmol^{-1} g $^{-1}$tissue min $^{-1}$
[3] Values in columns within each maturity stage followed by the same letter are not significantly different at the 0 05 level by LSD method
[4] Separate LSD test was used for this treatment.

irradiation on protein samples from AIS pink fruit was noted to be similar to that in samples from pink fruit reported earlier in Chapter 4 (Fig 4-12) Whereas proteins from irradiated mature green fruit at the high irradiation dose showed slightly lower levels than the control and the low irradiation dose on the first day following irradiation (Chapter 4 Fig 4-11) PG enzyme was not detectable in irradiated mature-green protein samples, which is in agreement with results reported in Chapter 3 (Fig 3 7), and is consistent with

the reported absence of PG in mature-green fruit (Hobson, 1964, Buescher and Tigchelaar, 1975, Grierson et al., 1987). There was, however, considerable reduction in the total activity of PG enzyme in irradiated pink protein sample compared to non-irradiated control samples (Table 5-8), which is consistent with results reported earlier in Chapter 3 (Fig. 3-8). Interestingly, irradiated pure PG samples at 1.0 ± 0.04 kGy showed a significant reduction in the enzyme activity compared to the non-irradiated control (Table 5-8). Irradiated protein samples in both mature-green and pink samples revealed no significant change in the activity of β-galactosidase enzyme relative to the control (Table 5-8).

Conclusions

The effect of irradiation on the texture and cell wall of tomato fruit is evident by the results presented here. Softening of tomato fruit is believed to be due to the degradation of cell wall components, mainly pectin fractions, evident by the increased solubility of water and chelator-soluble fractions, downshifting of Mr of pectins, and the increase yield in neutral sugars mainly arabinose and galactose. The effect of irradiation on neutral sugars was very drastic and severe, evident by the significant loss in total neutral sugars, and loss in galactose and arabinose associated with increase in galactose and either xylose or mannose, compared to the controls.

The dramatic effect in pectic fraction noted *in vitro* might give a clear example of the effect of irradiation *in vitro*, however, in comparing the effect of irradiation on either

system, we have to bare in mind the absence of a highly organized biological system in *in vitro* system

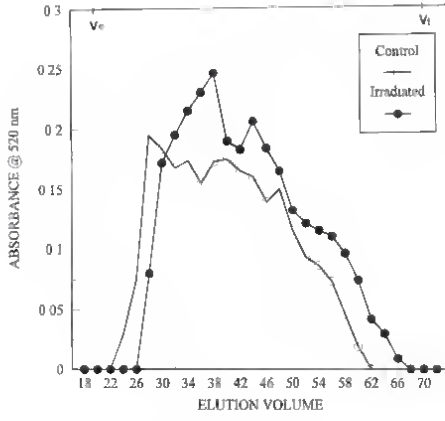


Figure 5-1 Sepharose CL-2B-300 profiles of H_2O-soluble pectic fractions derived from mature-green fruit AIS (non-BP-treated) and irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated) Vertical tick marks at the top of the profile indicate the elution positions of standards blue dextran 2,000 kDa (void volum,Vo) and glucose (total volume, Vt) Absorbance at 520 nm colorimetric determination of galacturonic equivalents

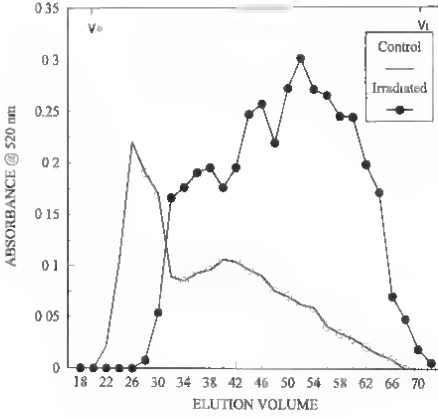


Figure 5-2 Sepharose CL-2B-300 profiles of H_2O-soluble pectic fractions derived from mature-green fruit AIS (BP treated) and irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 5-1

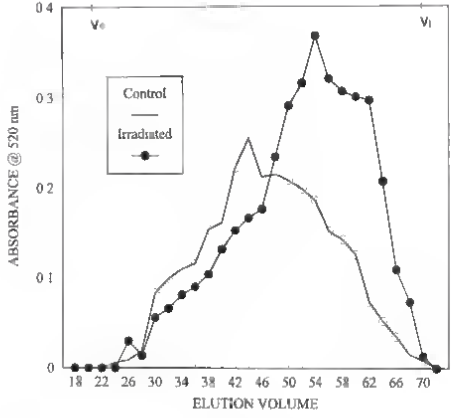


Figure 5-3 Sepharose CL 2B-300 profiles of H_2O-soluble pectic fractions derived from pink fruit AIS (non-BP-treated) and irradiated at 0 (control)or 1 0 ± 0 04 kGy (Irradiated) Vertical ticks (Vo & Vt) are as for Figure 5-1

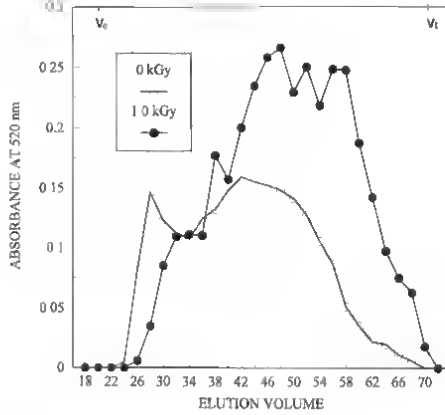


Figure 5-4 Sepharose CL-2B 300 profiles of H₂O-soluble pectic fractions derived from pink fruit AIS (BP-treated) and irradiated at 0 or 1 0 ± 0 04 kGy Vertical ticks (Vo & Vt) are as for Figure 5-1

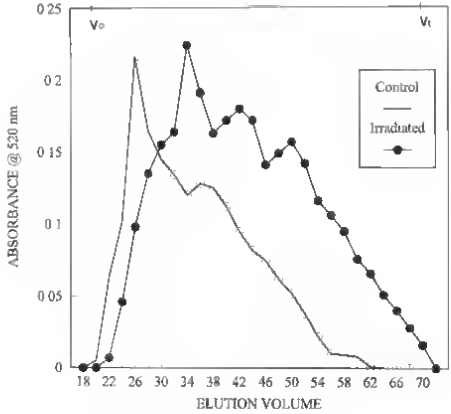


Figure 5-5 Sepharose CL-2B-300 profiles of CDTA-soluble pectic fractions derived from mature green fruit AIS (non BP-treated) and irradiated at 0 or 1 0 ± 0 04 kGy Vertical ticks (Vo & Vt) are as for Figure 5 1

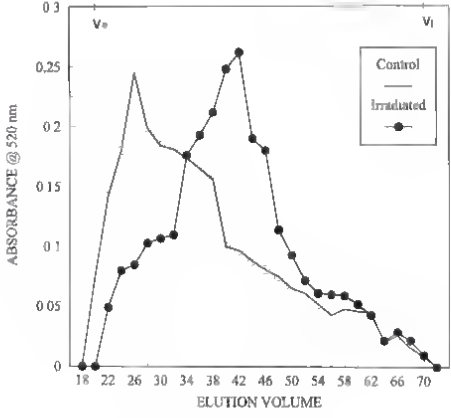


Figure 5-6 Sepharose CL-2B-300 profiles of CDTA-soluble pectic fractions derived from mature green fruit AIS (BP-treated) and irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 5-1

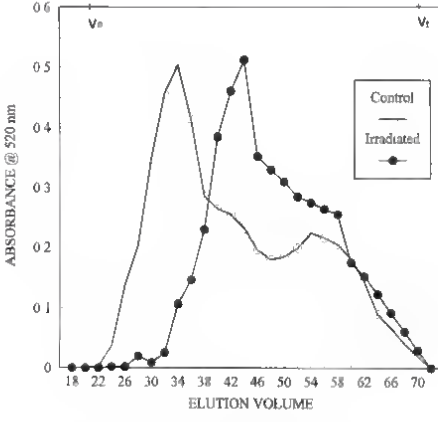


Figure 5-7 Sepharose CL-2B-300 profiles of CDTA soluble pectic fractions derived from pink fruit AIS (non-BP treated) and irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 5 1

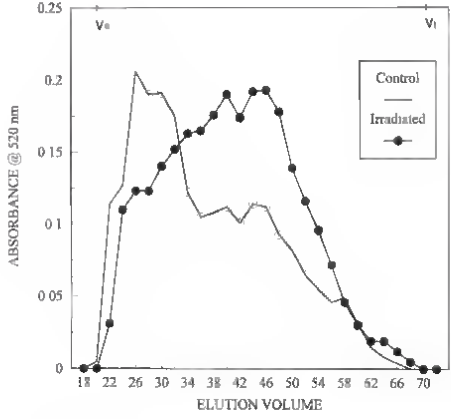


Figure 5-8 Sephurose CL-2B-300 profiles of CDTA-soluble pectic fractions derived from pink fruit AIS (BP-treated) and irradiated at 0 (control), or 1 0 ± 0 04 kGy (irradiated) Vertical ticks (Ve & Vt) are as for Figure 5-1

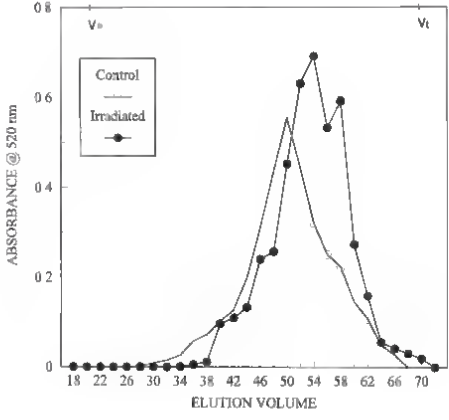


Figure 5-9 Sepharose CL 2B 300 profiles of H_2O-soluble pectic fractions derived from commericial pectins irradiated at 0 (control)or 1 0 ± 0 04 kGy (irradiated) Vertical ticks (Vo & Vt) are as for Figure 5-1

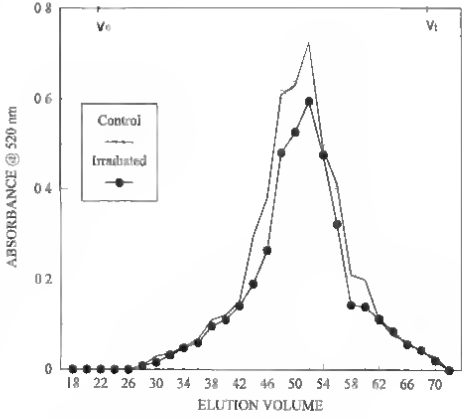


Figure 5-10.Sepharose CL-2B-300 profiles of H_2O soluble pectic fractions derived from polygalcturonic acid irradiated at 0 (control) or 1 0 ± 0 04 kGy (irradiated) Vertical tucks (Vo & Vt) are as for Figure 5-1

CHAPTER 6
SUMMARY AND CONCLUSIONS

Irradiation Induced Softening, Enzyme Alteration, and Cell Wall Textural Changes *In Vivo*

Sunny' tomato fruit at mature green and pink stages of development were subjected to ionizing irradiation from gamma-rays or X-rays. Whole fruit and pericarp tissue firmness, pericarp tissue cell wall enzyme activities, and electrolyte leakage were measured immediately following irradiation and during a 6- to 12- day post-irradiation storage period. An immediate loss of firmness was observed following irradiation treatments that advanced throughout the post-irradiation experimental period. Firmness loss was more pronounced in pericarp discs compared to the whole fruit and in mature-green compared to pink fruit. Enhanced electrolyte efflux was apparent in irradiated fruit of both maturity classes but was more pronounced in pink fruit. A significant reduction of PG activity was noted mainly in irradiated mature-green fruit, with a lesser effect on pink fruit. Increased activities of PME and β-galactosidase immediately following irradiation were followed by reductions at later stages in fruit of both maturity classes. The impact of irradiation on tomato fruit texture seems to involve cell wall modifications, altered cell wall enzyme activity, and loss of membrane function.

Irradiation-induced softening was associated with significant effects on the three enzymes tested (reduction in PG activity in both maturity classes, initial increases

followed by decreases in PME and β-galactosidase) Therefore, we conclude that the mechanism of irradiation induced softening might involve enzymes other than PG and/or occurred as a direct effect of irradiation on the structure of the cell wall A significant increase in the electrolyte efflux was observed in irradiated pink tomato fruit, whereas only slightly significant differences were noted in irradiated mature-green fruit We suggest that at least two different mechanisms are involved in the loss of firmness, one operative in both mature-green and pink fruit (cell wall related), and the other mainly in pink fruit (membrane related)

Irradiation Altered Cell Wall Matrix Properties

Polyuronides, hemicellulose, and selected proteins were examined in ripening tomato fruit to determine the effect of irradiation treatment on these wall components Uronic acid content, polyuronide solubility and M_w, neutral sugar analysis, and hemicellulose M_w changes were examined using alcohol insoluble solids (AIS) derived from pericarp tissue of irradiated fruit Lower levels of total uronic acid in the high irradiation treatments along with increased levels of H_2O-soluble polyuronides in both irradiation dose treatments, were observed in the irradiated mature-green and pink tomato fruit. Irradiated fruit also exhibited downshifts in the M_w of H_2O- and CDTA-soluble polyuronides Increased levels of CDTA soluble polyuronides were observed in irradiated mature-green fruit, whereas less pronounced changes were noted in irradiated pink fruit. Minimal alterations due to irradiation treatment were noted in the Na_2CO_3 soluble polyuronides, hemicelluloses and neutral sugars in both maturity stages

Levels of extractable proteins were lower in the irradiated mature-green fruit compared to control fruit within the first 24 hr following irradiation. However, increased protein levels were detected on the third day in the irradiated mature-green fruit relative to the protein levels detected within the first 24 hr following irradiation, which also exceeded the protein levels of the control. Thereafter, no significant changes in the protein levels in mature-green fruit were noted. Levels of proteins in pink irradiated fruit were higher relative to levels in the control for the first 3 days, followed by minimal changes thereafter. SDS-PAGE revealed the absence of PG enzyme in the irradiated mature-green fruit at the time when it was present in the non-irradiated fruit.

These results indicate that cell wall modifications occurred in both maturity stages, but mature-green fruit were more affected than pink fruit. The effect of irradiation on cell wall matrix polymers was proportional to the dose of irradiation. Irradiation affected the protein levels by upregulating and/ or downregulating specific proteins, including PG. The effect of irradiation was comprehensive, direct and/or indirect, and possibly acting in a random fashion affecting some of the cell wall features tested.

The effect of irradiation on the cell wall matrix polymers was direct and/ or indirect via mechanism(s) that affected the cell wall structure, resulting in the noted softening of tomato fruit. The decrease in the total uronic acid content in the higher irradiation dose treatments, the increased solubility and the downshifts in the M_r of the water- and CDTA- soluble polyuronides, along with slight influences on the neutral sugars and hemicellulose, indicate that the pectic fractions were the most affected by

irradiation. Therefore we assume that the degradation of the pectic fractions was the major event characterizing irradiation-induced tissue softening in tomato fruit

The changes noted in the proteins, on the other hand might be partially responsible for the disturbed pattern of normal ripening, resulting in the irregular ripening observed, in addition to other effects related to their contribution to the enhancement and/or regulation of tissue softening The effect of irradiation was more severe in mature-green than in pink fruit, indicating that the effect of irradiation on the younger tissue might have adversely affected the synthesis of proteins and enzymes that regulate the normal development of the fruit, in addition to the drastic effect on the cell wall structure

In Vitro Analysis

Alcohol insoluble solids (AIS) derived from 'Sunny' tomato fruit at the mature-green and pink stages of development were irradiated at 0 or 1.0 ± 0.04 kGy Irradiated plant materials were examined for irradiation-induced modifications to the cell wall.

Losses in total uronic acid and increased solubility of pectic fractions occurred in irradiated AIS preparations, indicating that irradiation induced extensive degradation of cell wall pectic polymers *in vitro* Differences in total uronic acid content were also noted between non-BP treated (enzymically active), and BP-treated (enzymically inactive) AIS This confirms the presumed direct effect of irradiation evident by the increased solubility and downshifts in the molecular weight of the enzymically inactive preparations Additionally, the effect is presumed to be due to the presence of non-cell

wall components to EtOH-washed samples from the AIS of mature-green fruit, while in AIS from pink fruit, enzymic reactions likely contributed to the differences

Considerable M_r downshifts occurred in the pectins of irradiated active and inactive AIS and commercial pectins. Cellulose was apparently not affected by irradiation. Neutral sugars were affected by irradiation as evident from the losses in total neutral sugars, losses in galactose and arabinose, and increases in glucose and other neutral sugars. Salt-extractable proteins increased in irradiated samples of AIS from mature-green and pink fruit, while PG decreased relative to levels in the non-irradiated controls. β-galactosidase was not affected by irradiation. Pure PG showed dramatically lower activity levels compared to the control.

The effect of irradiation on the texture and cell wall of tomato fruit is evident from the results presented here. Softening of tomato fruit is believed to be due to the degradation of cell wall components, mainly in the pectin fractions as evidenced by the increased solubility of water and chelator-soluble fractions, downshifting of M_r of pectins, and the increased yield of neutral sugars, mainly arabinose and galactose. The effect of irradiation on neutral sugars was very drastic, evident by the significant loss in total neutral sugars, and losses in galactose and arabinose associated with increases in glucose and either xylose or mannose, compared to the controls.

There were no apparent differences between pericarp and locular gel tissue in terms of their response to irradiation. Both types of tissue exhibited an increase in polyuronide solubility and downshifting of the M_r, although the downshifting of M_r in pericarp tissue was slightly more pronounced. However, since pericarp tissue contains

proportionally more cell wall materials than locular gel tissue, it is considered to have more impact on the softening of the fruit

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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